

CORRELATION OF *rpsU* GENE SEQUENCE CLUSTERS AND BIOCHEMICAL PROPERTIES, GC–MS SPECTRA AND RESISTANCE PROFILES OF CLINICAL *BURKHOLDERIA* spp. ISOLATES

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This study assessed the variation of phenotypic features of clinical isolates of *Burkholderia* spp. from common *rpsU* gene sequence clusters.

A total of 41 clinical *Burkholderia* spp. isolates from German mucoviscidosis patients was subjected to *rpsU* gene sequencing. Biochemical assessment included the API systems 20 NE and 50 CHE as well as the Micronaut NF system. Fatty acid patterns were assessed using gas chromatography–mass spectrometry (GC–MS). Broth microdilution was used to identify minimum inhibitory concentrations.

Five *rpsU* gene sequence clusters comprised more than one clinical isolate. Altogether, assignments to three species and seven clusters comprising more than one *Burkholderia* species were performed. Inhomogeneity of biochemical reactions within the clusters ranged from 0/28 to 45/50 reactions. The standard deviation for fatty acid distributions ranged from 0% to 11.5%. Minimum inhibitory concentrations within the clusters showed a wide variation but only minor differences between the clusters.

Broad variations within identified *rpsU* gene sequence clusters regarding biochemical reactions, fatty acid patterns, and resistance patterns of clinical *Burkholderia* spp. isolates make the application of *rpsU* gene sequence analysis as a stand-alone procedure for discriminations within the *Burkholderia cepacia* complex unreliable.

Keywords: *Burkholderia*, *rpsU*, ribosomal protein S21, typing, GC–MS, resistance, biochemical differentiation, clinical isolates

Introduction

Sequence analysis of the *rpsU* gene has been assessed as a potential tool for the discrimination of *Burkholderia* spp. beyond the genus level in previous studies [1–3]. The procedure allows for a discrimination of the environmental commensal species *Burkholderia thailandensis* from the phylogenetically closely related pathogens *Burkholderia mallei* and *Burkholderia pseudomallei* [2]. Further, *rpsU* gene sequencing can be used for a discrimination of this *B. pseudomallei* complex from other facultatively pathogenic or just environmental *Burkholderia* spp. The sequences of reference strains grouped in four major clusters. Within these clusters, however, differentiation on species level was found to be unreliable [3].

While the discriminative power of *rpsU* gene sequencing has been well characterized with sequences from reference strains [3], respective analyses for clinical *Burkholderia* isolates are missing so far. Therefore, clinical isolates that had been biochemically characterized as *Burkholderia cepacia* by API 20 NE (BioMérieux, Marcy-l’Étoile, France) were subjected to *rpsU* gene clustering. The isolates from the resulting clusters were analyzed for homogeneity or heterogeneity regarding biochemical features, composition of fatty acids, and resistance patterns.

By doing so, it was assessed whether the *rpsU* gene clusters indeed indicate clusters of phenotypically related *Burkholderia* spp. isolates, which might be of use for diagnostic purposes, or whether they are clustered without further relevance for the diagnostic microbiological laboratory.

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Methods

Clinical isolates

In the 1990s, a total of 41 clinical *Burkholderia* spp. isolates from different mucoviscidosis patients were collected in Munich, Germany. All strains were subjected to biochemical differentiation by API 20 NE (BioMérieux), resulting in the preliminary diagnosis of *B. cepacia*. Considering the low discriminative potential of API 20 NE within the *Burkholderia* genus, all respective isolates were subjected to further molecular and phenotypic assessment as detailed below. To avoid the inclusion of copy strains, only one isolate per patient was used for further assessments. Due to events of losing strains during subculture passages, not all assessments could be performed for all strains.

rpsU gene sequence clustering

All 41 strains were subjected to *rpsU* gene PCR and sequencing as described previously [2, 3] to obtain 169-base-pair sequences. Due to a required minimum of 200 bp, the obtained short sequence fragments could not be deposited at NCBI GenBank and are thus presented in the electronic supplementary material (*Supporting Information 1*).

All obtained sequences were clustered with the previously presented sequences from *Burkholderia* spp. reference strains [3] and well-characterized strains of the *B. pseudomallei* complex [2] using the software BioNumerics 7.1 (Applied Maths, Sint-Martens-Latem, Belgium).

Species assignment was based on closest proximity to sequences of a reference strain and a variance to the reference sequence of <1%. In case of two “out-standers”, identification was based on a NCBI GenBank search with a detected sequence identity of ≥99%. In case of more than one match, all matching species were indicated, divided by the acronym “DD” (differential diagnosis).

Biochemical characterization

Next to the abovementioned API 20 NE (BioMérieux), the strains were analyzed with the API 50 CHE system (BioMérieux) and the Micronaut NF ID system (MERLIN Gesellschaft für mikrobiologische Diagnostika mbH, Bornheim-Hersel, Germany). The diagnostic procedure was performed as described by the manufacturers.

Characterization of fatty acid patterns

Bacterial fatty acids of the *Burkholderia* spp. isolates were assessed using GC–MS (gas chromatography–mass spectrometry) with a trimethylsulfoniumhydroxide (TMSH)-based procedure as previously described [4]. In short, saponification of the fatty acids of the bacteria is driven

by TMSH, resulting in TMSH salts. These salts are split at 260 °C in the injector block of the gas chromatograph into dimethylsulfide and the methyl esters of the respective fatty acids. The methyl esters are afterwards divided by gas chromatography.

Characterization of resistance patterns

Susceptibility of the assessed strains towards antibiotic drugs was assessed by traditional broth microdilution testing to identify the minimum inhibitory concentration. Broth microdilution was performed in customer-designed broth microdilution plates (MERLIN Gesellschaft für mikrobiologische Diagnostika mbH). The assessed antibiotic drugs comprised both substances for Gram-positive and Gram-negative bacteria, i.e., amikacin, netilmicin, tobramycin, streptomycin, kanamycin, neomycin, spectinomycin, penicillin G, amoxicillin, azlocillin, piperacillin, ticarcillin, oxacillin, ampicillin sulbactam, sulbactam, cefaclor, cefixime, apramycin, cefpodoxime, loracarbef, cefdinir, cefetamet, ceftibuten, cefazolin, cefotaxime, ceftriaxone, cefotixin, ceftazidime, cefoperazone, cefotiam, mezlocillin, aztreonam, imipenem, amoxicillin clavulanate, meropenem, piperacillin tazobactam, biapenem, ribostamycin, pipemidic acid, norfloxacin, ofloxacin, ciprofloxacin, enoxacin, fleroxacin, pefloxacin, sparfloxacin, erythromycin, clindamycin, lincomycin, roxithromycin, clarithromycin, azithromycin, quinopristin/dalfopristin, luitomycin A, tetracycline, doxycycline, minocycline, chloramphenicol, sulfamethoxazole, nitrofurantoin, cotrimoxazole, trimethoprim, fosfomycin, vancomycin, rifampicin, teicoplanin, fusidic acid, cefepime, dalfopristin, and quinopristin.

Ethics

No ethical clearance was necessary because this study did not include patients, patient data, or patient materials.

Results

Observed *rpsU* gene sequence clusters

In eight out of 41 *Burkholderia* spp. isolates, *rpsU* gene sequence clustering allowed for an assignment on species level. The resulting species comprised *B. cepacia* ($n = 5$), *Burkholderia multivorans* ($n = 2$), and *Burkholderia glumae* ($n = 1$). For the remaining 33 out of 41 *Burkholderia* spp., no unambiguous assignment at species level was possible, resulting in seven clusters *B. multivorans* DD *Burkholderia caryophili* ($n = 25$), *B. cepacia* DD *Burkholderia vietnamensis* ($n = 2$), *B. cepacia* DD *Burkholderia cenocepacia* ($n = 2$), *B. cepacia* DD *B. vietnamensis* DD *B. cenocepacia* DD *Burkholderia ambifaria* ($n = 1$), *B. cepacia* DD *B. vietnamensis* DD *Burkholderia anthina*

DD *B. ambifaria* ($n = 1$), *Burkholderia gladioli* DD *Burkholderia cocovenenans* ($n = 1$), and *Burkholderia caribensis* DD *Burkholderia hospita* ($n = 1$), respectively.

Adjusted to the previously suggested *rpsU* gene clusters [3], two isolates could be assigned to cluster *rpsU-I*, zero isolates to *rpsU-II*, 36 isolates to *rpsU-III*, and one isolate to *rpsU-IV*, respectively. The sequences of two isolates, one strain *B. multivorans* and one strain *B. cepacia* DD *B. vietnamiensis* DD *B. ambifaria* DD *B. cenocepacia*, could not be assigned to any of the proposed clusters [3] and had to be identified by NCBI BLAST search.

Biochemical features within the observed clusters

API 20 NE profiles were available for 40 out of 41 isolates. For one isolate *B. multivorans* DD *B. caryophili*, the profile had been lost and could not be reassessed because the isolate had died during passage. Data from API 50 CHE and Micronaut NF could be assessed for 38 out of 41 isolates.

Table 1. API 20 NE results

Species/species groups	Reduction of potassium nitrate (%)	Indol production from tryptophan (%)	Glucose fermentation (%)	Arginine hydrolysis (%)	Urea hydrolysis (%)
<i>B. multivorans</i> DD <i>B. caryophili</i> ($n = 24$)	91.7	0	13	0	4.2
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> ($n = 2$)	0	0	0	0	0
<i>B. cepacia</i> DD <i>B. cenocepacia</i> ($n = 2$)	100	0	0	0	0
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. cenocepacia</i> DD <i>B. ambifaria</i> ($n = 1$)	0	0	0	0	0
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. anthina</i> DD <i>B. ambifaria</i> ($n = 1$)	100	0	0	0	0
<i>B. multivorans</i> ($n = 2$)	100	0	0	0	0
<i>B. gladioli</i> DD <i>B. cocovenenans</i> ($n = 1$)	100	0	100	0	0
<i>B. glumiae</i> ($n = 1$)	100	0	0	0	0
<i>B. cepacia</i> ($n = 5$)	20	0	0	0	0
<i>B. caribensis</i> DD <i>B. hospita</i> ($n = 1$)	0	0	0	0	0
Species/species groups	Aesculin hydrolysis (%)	Gelatin hydrolysis (%)	p-Nitrophenyl-β-D-galactopyranoside hydrolysis (%)	Glucose assimilation (%)	Arabinose assimilation (%)
<i>B. multivorans</i> DD <i>B. caryophili</i> ($n = 24$)	75	8.3	45.8	100	91.7
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> ($n = 2$)	50	50	50	100	100
<i>B. cepacia</i> DD <i>B. cenocepacia</i> ($n = 2$)	100	50	100	100	100

The detailed biochemical profiles of the API 20 NE, API 50 CHE, and Micronaut NF systems are depicted in Tables 1–3, respectively. Homogeneity of the biochemical patterns was assessed for all clusters with more than one isolate.

Regarding API 20 NE, inhomogeneity was observed for 8/20 reactions in the *B. multivorans* DD *B. caryophili* cluster, for 3/20 reactions in the *B. cepacia* DD *B. vietnamiensis* cluster, for 1/20 reactions in the *B. cepacia* DD *B. cenocepacia* cluster, for 3/20 reactions in the *B. multivorans* cluster, and for 4/20 reactions in the *B. cepacia* cluster (Table 1).

Regarding API 50 CHE, inhomogeneity was observed for 45/50 reactions in the *B. multivorans* DD *B. caryophili* cluster, for 3/50 reactions in the *B. cepacia* DD *B. vietnamiensis* cluster, for 24/50 reactions in the *B. cepacia* DD *B. cenocepacia* cluster, for 25/50 reactions in the *B. multivorans* cluster, and for 31/50 reactions in the *B. cepacia* cluster (Table 2).

Regarding Micronaut NF, inhomogeneity was observed for 11/28 reactions in the *B. multivorans* DD *B. caryophili*

Table 1. (cont'd)

Species/species groups	Aesculin hydrolysis (%)	Gelatin hydrolysis (%)	p-Nitrophenyl-β-D-galactopyranoside hydrolysis (%)	Glucose assimilation (%)	Arabinose assimilation (%)
<i>B. cepacia</i> DD	100	100	100	100	100
<i>B. vietnamiensis</i> DD					
<i>B. cenocepacia</i> DD					
<i>B. ambifaria</i> (n = 1)					
<i>B. cepacia</i> DD	100	100	0	100	100
<i>B. vietnamiensis</i> DD					
<i>B. anthina</i> DD <i>B. ambifaria</i> (n = 1)					
<i>B. multivorans</i> (n = 2)	50	0	50	100	100
<i>B. gladioli</i> DD	0	0	100	100	0
<i>B. cocovenenans</i> (n = 1)					
<i>B. glumae</i> (n = 1)	100	0	100	100	100
<i>B. cepacia</i> (n = 5)	100	60	40	100	100
<i>B. caribensis</i> DD <i>B. hospita</i> (n = 1)	100	0	100	100	100
Species/species groups	Mannose assimilation (%)	Mannitol assimilation (%)	N-acetyl-glucosamine assimilation (%)	Maltose assimilation (%)	Gluconate assimilation (%)
<i>B. multivorans</i> DD	100	100	91.7	0	100
<i>B. caryophili</i> (n = 24)					
<i>B. cepacia</i> DD	100	50	100	100	100
<i>B. vietnamiensis</i> (n = 2)					
<i>B. cepacia</i> DD	100	100	100	0	100
<i>B. cenocepacia</i> (n = 2)					
<i>B. cepacia</i> DD	100	100	100	0	100
<i>B. vietnamiensis</i> DD					
<i>B. cenocepacia</i> DD					
<i>B. ambifaria</i> (n = 1)					
<i>B. cepacia</i> DD	100	100	100	0	100
<i>B. vietnamiensis</i> DD					
<i>B. anthina</i> DD <i>B. ambifaria</i> (n = 1)					
<i>B. multivorans</i> (n = 2)	100	100	50	0	100
<i>B. gladioli</i> DD	100	100	100	0	100
<i>B. cocovenenans</i> (n = 1)					
<i>B. glumae</i> (n = 1)	100	100	100	0	100
<i>B. cepacia</i> (n = 5)	100	100	100	40	100
<i>B. caribensis</i> DD <i>B. hospita</i> (n = 1)	100	100	100	100	100
Species/species groups	Caprate assimilation (%)	Adipate assimilation (%)	Malate assimilation (%)	Citrate assimilation (%)	Phenyl-acetat assimilation (%)
<i>B. multivorans</i> DD	100	100	100	100	100
<i>B. caryophili</i> (n = 24)					
<i>B. cepacia</i> DD	100	100	100	100	100
<i>B. vietnamiensis</i> (n = 2)					
<i>B. cepacia</i> DD	100	100	100	100	100
<i>B. cenocepacia</i> (n = 2)					
<i>B. cepacia</i> DD	100	100	100	100	100
<i>B. vietnamiensis</i> DD					
<i>B. cenocepacia</i> DD					
<i>B. ambifaria</i> (n = 1)					

Table 1. (cont'd)

Species/species groups	Caprate assimilation (%)	Adipate assimilation (%)	Malate assimilation (%)	Citrate assimilation (%)	Phenyl-acetate assimilation (%)
<i>B. cepacia</i> DD	100	100	100	100	100
<i>B. vietnamiensis</i> DD					
<i>B. anthina</i> DD <i>B. ambifaria</i> (n = 1)					
<i>B. multivorans</i> (n = 2)	100	100	100	100	100
<i>B. gladioli</i> DD	100	100	100	100	100
<i>B. cocovenenans</i> (n = 1)					
<i>B. glumae</i> (n = 1)	100	100	100	100	100
<i>B. cepacia</i> (n = 5)	100	100	100	100	100
<i>B. caribensis</i> DD <i>B. hospita</i> (n = 1)	0	100	100	100	100

cluster, for 3/28 reactions in the *B. cepacia* DD *B. vietnamiensis* cluster, for 2/28 reactions in the *B. cepacia* DD *B. cenocepacia* cluster, for 0/28 reactions in the *B. multivorans* cluster, and for 7/28 reactions in the *B. cepacia* cluster after 24 h. Of note, an increase in the percentage of isolates showing *N*-acetyl-glucosamine assimilation was observed after 48 h (*Table 3*).

Composition of fatty acid patterns within the observed clusters

The patterns of fatty acid distribution of the *Burkholderia* spp. strains are shown in *Table 4*. The standard deviations for the individual tested fatty acids for the *B. multivorans* DD *B. caryophili* cluster ranged from 0% to 11.5%, for the *B. cepacia* DD *B. vietnamiensis* cluster from 0% to 5.5%, for the *B. cepacia* DD *B. cenocepacia* cluster from 0% to 2.7%, and for the *B. cepacia* cluster from 0% to 3.77% (*Table 4*).

Compositions of resistance patterns within the observed clusters

Details on the detected minimum inhibitory concentration of the tested antimicrobial substances are shown in the electronic supplementary material (*Supporting Information 2*). Varieties of up to 17 serial dilution steps were observed for the *rpsU* gene sequence clusters.

In direct comparison of the *B. multivorans* DD *B. caryophili* cluster and the *B. cepacia* cluster, lower minimum inhibitory concentrations were observed for the aminoglycosides gentamycin, tobramycin, streptomycin, kanamycin, neomycin, and spectinomycin in the *B. cepacia* cluster. Among the β -lactam antibiotics, the lowest minimum inhibitory concentrations were observed for oxacillin. For all tested fluoroquinolones, a broad range of minimum inhibitory concentrations was measured (*Supporting Information 2*).

Discussion

The *rpsU* gene codes for the ribosomal protein S21 [2, 3]. Its role in the biochemical processes of the bacterial cell is a topic of ongoing research. A previous study indicated a role of ribosomal protein S21 for motility and biofilm formation of *Bacillus subtilis* [5]. Research with *Helicobacter pylori* suggests a potential role of *rpsU* gene mutations for metronidazole resistance [6]. In *Listeria monocytogenes* strains, influence of *rpsU* gene variants on stress resistance in vital cells could be demonstrated [7].

In the field of diagnostic microbiology, *rpsU* gene sequencing has been assessed for its discriminatory potential in the field of *Burkholderia* spp. differentiation. As previously demonstrated, the procedure is suited to discriminate highly pathogenic *B. mallei*/*B. pseudomallei* [8] from environmental *B. thailandensis* strains within the *B. pseudomallei* complex [2]. The respective sequence cluster of analyzed strains of the *B. pseudomallei* complex was later shown to form a distinct cluster *rpsU-II* that can be well discriminated from other *Burkholderia* spp. [3].

Within other *rpsU* gene sequence clusters, however, sequence comparison of well characterized reference strains showed high similarity, not allowing for reliable discrimination on species level within the *B. cepacia* complex [3]. The respective analysis, however, included reference strains only and did not include phenotypic features of the strains.

Therefore, a potential correlation of *rpsU* gene sequence clustering and phenotypical properties of clinical *Burkholderia* spp. isolates remained completely unclear so far. To address this topic, clinical *Burkholderia* spp. strains from mucoviscidosis patients that had been identified as "*B. cepacia*" by API 20 NE were included in the study. The lacking reliability of biochemical assessments for the discrimination of *Burkholderia* spp. on species level has been repeatedly demonstrated [9–12]. Accordingly, the isolates were subjected to *rpsU* gene sequencing for further discrimination.

Table 2. API 50 CHE results

Species/species groups	Control (%)	Glycerol (%)	Erythritol (%)	D-Arabinose (%)	L-Arabinose (%)	Ribose (%)	D-Xylose (%)	L-Xylose (%)	Adonitol (%)	β -Methyl-xyloside (%)
<i>B. multivorans</i> DD	4.2	58.3	16.7	62.7	79.2	29.2	37.5	33.3	58.3	20.8
<i>B. caryophili</i> (n = 24)										
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamensis</i> (n = 2)										
<i>B. cepacia</i> DD	0	0	0	0	50	0	50	0	0	0
<i>B. cenocepacia</i> (n = 2)										
<i>B. cepacia</i> DD	0	0	0	0	100	0	100	0	0	0
<i>B. vietnamensis</i> DD										
<i>B. cenocepacia</i> DD										
<i>B. ambifaria</i> (n = 1)										
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamensis</i> DD										
<i>B. anthina</i> DD										
<i>B. ambifaria</i> (n = 1)										
<i>B. multivorans</i> (n = 2)	0	50	0	100	100	0	50	50	50	50
<i>B. gladioli</i> DD	0	100	0	0	0	0	0	0	0	0
<i>B. cocovenans</i> (n = 1)										
<i>B. cepacia</i> (n = 5)	0	20	0	60	40	0	80	20	60	40
Species/species groups	Galactose (%)	D-Glucose (%)	D-Fructose (%)	D-Mannose (%)	L-Sorbitose (%)	Rhamnose (%)	Dulcitol (%)	Inositol (%)	Mannitol (%)	Sorbitol (%)
<i>B. multivorans</i> DD	100	100	75	100	8.3	20.8	62.5	70.8	58.3	75
<i>B. caryophili</i> (n = 24)										
<i>B. cepacia</i> DD	100	100	0	0	0	0	0	0	0	0
<i>B. vietnamensis</i> (n = 2)										
<i>B. cepacia</i> DD	50	50	0	0	0	0	50	0	50	0
<i>B. cenocepacia</i> (n = 2)										

Table 2. (cont'd)

Species/species groups	Galactose (%)	D-Glucose (%)	D-Fructose (%)	D-Mannose (%)	L-Sorbitose (%)	Rhamnose (%)	Dulcitol (%)	Inositol (%)	Mannitol (%)	Sorbitol (%)
<i>B. cepacia</i> DD	100	100	100	100	100	0	100	100	0	100
<i>B. vietnamiensis</i> DD										
<i>B. cenocepacia</i> DD										
<i>B. ambifaria</i> (<i>n</i> = 1)										
<i>B. cepacia</i> DD	100	100	0	100	0	100	0	0	100	0
<i>B. vietnamiensis</i> DD										
<i>B. anthina</i> DD										
<i>B. ambifaria</i> (<i>n</i> = 1)										
<i>B. multivorans</i>	100	100	50	50	0	50	50	100	50	100
(<i>n</i> = 2)										
<i>B. gladioli</i> DD	100	100	100	100	0	0	100	100	100	100
<i>B. cocovenans</i>										
(<i>n</i> = 1)										
<i>B. cepacia</i> (<i>n</i> = 5)	100	100	60	60	0	0	60	40	60	60
<i>B. cepacia</i> DD										
<i>B. caryophili</i>										
(<i>n</i> = 24)										
<i>B. multivorans</i> DD	12.5	20.8	12.5	8.3	16.7	54.2	4.2	54.2	70.8	83.3
<i>B. vietnamiensis</i>										
(<i>n</i> = 2)										
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	50	50
<i>B. cenocepacia</i>										
(<i>n</i> = 2)										
<i>B. cepacia</i> DD	0	0	50	0	50	100	50	50	50	50
<i>B. vietnamiensis</i> DD										
<i>B. cenocepacia</i> DD										
<i>B. ambifaria</i> (<i>n</i> = 1)										
<i>B. cepacia</i> DD	0	0	0	0	0	100	0	0	0	0
<i>B. vietnamiensis</i> DD										
<i>B. anthina</i> DD										
<i>B. ambifaria</i> (<i>n</i> = 1)										
<i>B. multivorans</i>	0	50	50	0	50	100	50	50	50	50
(<i>n</i> = 2)										

Table 2. (cont'd)

Species/species groups	α -Methyl-D-mannoside (%)	a-Methyl-D-glucoside (%)	N-acetyl glucosamine (%)	Amrygdaline (%)	Arbutine (%)	Esculin (%)	Salicine (%)	Celllobiose (%)	Maltose (%)	Lactose (%)
<i>B. gladioli</i> DD	0	0	0	0	0	0	0	100	0	100
<i>B. cocovenans</i> (n = 1)										
<i>B. cepacia</i> (n = 5)	20	20	20	0	20	80	20	80	40	60
Species/species groups	Melibiose (%)	Saccharose (%)	Trehalose (%)	Inuline (%)	Melizitose (%)	D-Raffinose (%)	Amidon (%)	Glycogene (%)	Xylitol (%)	β -Gentibiose (%)
<i>B. multivorans</i> DD	16.7	12.5	37.5	16.7	12.5	12.5	12.5	8.3	33.3	41.7
<i>B. caryophili</i> (n = 24)										
<i>B. cepacia</i> DD <i>B. vietnamensis</i> (n = 2)	0	0	0	0	0	0	0	0	0	0
<i>B. cepacia</i> DD <i>B. cenocepacia</i> (n = 2)	0	50	50	0	0	50	50	50	50	50
<i>B. cepacia</i> DD <i>B. vietnamensis</i> DD	0	100	0	100	0	100	0	100	100	0
<i>B. cenocepacia</i> DD										
<i>B. ambifaria</i> (n = 1)										
<i>B. cepacia</i> DD <i>B. vietnamensis</i> DD	0	0	100	0	0	0	0	0	0	100
<i>B. anthina</i> DD										
<i>B. ambifaria</i> (n = 1)										
<i>B. multivorans</i> (n = 2)	0	0	50	0	0	50	0	0	50	50
<i>B. gladioli</i> DD <i>B. cocovenans</i> (n = 1)	0	0	100	0	0	0	0	0	0	100
<i>B. cepacia</i> (n = 5)	20	100	0	20	0	0	20	0	0	0
Species/species groups	D-Turanose (%)	D-Lyxose (%)	D-Tagatose (%)	L-Fucose (%)	D-Arabinol (%)	L-Arabinol (%)	Gluconate (%)	2-Ceto-glucconate (%)	5-Ceto-glucconate (%)	
<i>B. multivorans</i> DD	8.3	37.5	29.2	100	29.2	25	25	8.3	0	12.5
<i>B. caryophili</i> (n = 24)										

Table 2. (cont'd)

Species/species groups	D-Turanose (%)	D-Lyxose (%)	D-Tagatose (%)	D-Fucose (%)	L-Fucose (%)	D-Arabinol (%)	L-Arabinol (%)	Gluconate (%)	2-Ceto-gluconate (%)	5-Ceto-gluconate (%)
<i>B. cepacia</i> DD	0	0	0	50	0	0	0	0	0	0
<i>B. vietnamiensis</i> (n = 2)										
<i>B. cepacia</i> DD <i>B. cenocepacia</i> (n = 2)	0	50	0	100	50	50	50	0	0	50
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. cenocepacia</i> DD <i>B. ambifaria</i> (n = 1)	100	100	100	100	0	0	0	100	0	100
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. anthina</i> DD <i>B. ambifaria</i> (n = 1)	0	0	0	100	100	50	100	0	100	0
<i>B. multivorans</i> (n = 2)	0	0	0	100	50	100	50	50	0	0
<i>B. gladioli</i> DD <i>B. cocovenans</i> (n = 1)	0	100	0	0	0	100	0	0	0	0
<i>B. cepacia</i> (n = 5)	20	40	40	80	40	20	0	0	0	0

The 41 isolates were assigned to three species and seven clusters of species groups, not allowing for an unambiguous assignment. This phenomenon confirms the results as observed for the reference strains in the previous assessment [3], limiting the diagnostic value of *rpsU* gene sequencing on species level. Interestingly, two out-standers were observed that did not match with any of the described *rpsU* gene sequence clusters [3] at all. This observation challenges the assumption of relative genetic stability of the *rpsU* gene region within distinct *Burkholderia* spp. [13]. Studies with larger sample counts might demonstrate further variability, making its diagnostic potential even less valuable.

Biochemical data of species or species group assignments that comprise more than one strain suggest a broad phenotypic heterogeneity. This phenomenon was observed for all three assessed biochemical identification systems. Accordingly, it is highly likely that different species were assigned to the same *rpsU* gene sequence cluster. This was not only the case for assignments to species groups comprising more than one *Burkholderia* spp. The *B. cepacia* cluster and the *B. multivorans* cluster demonstrated considerable biochemical heterogeneity as well. This suggests incorrect assignments if just the *rpsU* gene sequence is considered.

The same applies to the results of the fatty acid assessments. Cluster-dependent standard deviations of up to 11.5% within a certain *rpsU* gene sequence cluster express a variability that does not speak in favor of a correct assignment on species level [4].

The broad variability of the genetic resistance patterns is interesting but does not provide much information with regards to the source of the isolates. For patients with a medical history of mucoviscidosis, numerous previous antibiotic therapies have to be assumed. No patient specific data were available for this study. Nevertheless, selection of resistant strains in mucoviscidosis patients under repeated antibiotic pressure is highly likely, potentially accounting for the broad spectrum of minimum inhibitory concentrations of fluoroquinolones. Of note, the obvious shift in the resistance patterns for aminoglycosides

Table 3. Micronaut NF results

Species/species groups	Oxidase (%)	Indole (%)	Esculin (%)	Urease (%)	Ornithin-decarboxylase (%)	Arginin-dihydrolase (%)	Glucose fermentation (%)	Saccarose fermentation (%)
<i>B. multivorans</i> DD	100	0	0	0	4.2	0	100	8.3
<i>B. caryophili</i> (<i>n</i> = 24)								
<i>B. cepacia</i> DD	100	0	0	0	50	0	100	50
<i>B. vietnamiensis</i>								
(<i>n</i> = 2)								
<i>B. cepacia</i> DD	100	0	0	0	50	0	100	50
<i>B. cenocepacia</i>								
(<i>n</i> = 2)								
<i>B. cepacia</i> DD	100	0	0	0	0	0	100	100
<i>B. vietnamiensis</i> DD								
<i>B. cenocepacia</i> DD								
<i>B. ambifaria</i> (<i>n</i> = 1)								
<i>B. cepacia</i> DD	100	0	0	0	100	0	0	0
<i>B. vietnamiensis</i> DD								
<i>B. anthina</i> DD								
<i>B. ambifaria</i> (<i>n</i> = 1)								
<i>B. multivorans</i> (<i>n</i> = 1)	100	0	0	0	0	0	100	0
<i>B. cepacia</i> (<i>n</i> = 5)	100	0	0	0	0	0	80	80
<i>B. caribensis</i> DD	100	0	0	0	0	0	0	0
<i>B. hospita</i> (<i>n</i> = 1)								
Species/species groups	Glucose assimilation (%)	Mannose assimilation (%)	Maltose assimilation (%)	<i>N</i> -acetyl-glucosamine assimilation (%)	Mannitol assimilation (%)	Gluconate assimilation (%)	Hydroxybutyric acid assimilation (%)	Lactate assimilation (%)
<i>B. multivorans</i> DD	87.5	100	0	4.2/95.8*	100	100	100	25
<i>B. caryophili</i> (<i>n</i> = 24)								
<i>B. cepacia</i> DD	50	100	100	100	0	100	100	100
<i>B. vietnamiensis</i>								
(<i>n</i> = 2)								
<i>B. cepacia</i> DD	100	100	0	100	100	100	100	100
<i>B. cenocepacia</i>								
(<i>n</i> = 2)								
<i>B. cepacia</i> DD	100	100	0	100	100	100	100	100
<i>B. vietnamiensis</i> DD								
<i>B. cenocepacia</i> DD								
<i>B. ambifaria</i> (<i>n</i> = 1)								

Table 3. (cont'd)

Species/species groups	Glucose assimilation (%)	Mannose assimilation (%)	Maltose assimilation (%)	<i>N</i> -acetyl-glucosamine assimilation (%)	Mannitol assimilation (%)	Gluconate assimilation (%)	Hydroxybutyric acid assimilation (%)	Lactate assimilation (%)
<i>B. cepacia</i> DD	100	0	0	100	100	100	100	100
<i>B. vietnamiensis</i> DD								
<i>B. anthina</i> DD								
<i>B. ambifaria</i> (<i>n</i> = 1)								
<i>B. multivorans</i> (<i>n</i> = 1)	0	100	0	0	100	100	100	0
<i>B. cepacia</i> (<i>n</i> = 5)	100	100	0	100	100	100	100	100
<i>B. caribensis</i> DD	100	100	0	100	100	100	100	100
<i>B. hospita</i> (<i>n</i> = 1)								
Species/species groups	Adipate assimilation (%)	Suberate assimilation (%)	Malate assimilation (%)	Phenylacetic acid assimilation (%)	Histidine assimilation (%)	α -Nitrophenyl- β -galactosidase (%)	Phospholipase (%)	Phosphodiesterase (%)
<i>B. multivorans</i> DD	100	100	100	100	100	20.8	95.8	4.2
<i>B. caryophili</i> (<i>n</i> = 24)								
<i>B. cepacia</i> DD	100	100	100	100	100	0	100	0
<i>B. vietnamiensis</i> (<i>n</i> = 2)								
<i>B. cepacia</i> DD	100	100	100	100	100	0	100	0
<i>B. cenocepacia</i> (<i>n</i> = 2)								
<i>B. cepacia</i> DD	100	100	100	100	100	0	100	0
<i>B. vietnamiensis</i> DD								
<i>B. cenocepacia</i> DD								
<i>B. ambifaria</i> (<i>n</i> = 1)								
<i>B. cepacia</i> DD	100	100	100	100	100	0	100	0
<i>B. vietnamiensis</i> DD								
<i>B. anthina</i> DD								
<i>B. ambifaria</i> (<i>n</i> = 1)								
<i>B. multivorans</i> (<i>n</i> = 1)	100	100	100	100	100	0	100	0
<i>B. cepacia</i> (<i>n</i> = 5)	80	100	100	80	100	40	80	0
<i>B. caribensis</i> DD	0	100	100	0	0	0	100	0
<i>B. hospita</i> (<i>n</i> = 1)								

Table 3. (cont'd)

Species/species groups	Psolinamidase (%)	Maltosidase (%)	Chitinase (%)	Acid phosphatase (%)
<i>B. multivorans</i> DD <i>B. caryophili</i> (<i>n</i> = 24)	4.2	0	12.5	33.3
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> (<i>n</i> = 2)	100	0	100	50
<i>B. cepacia</i> DD <i>B. cenocapsacia</i> (<i>n</i> = 2)	0	0	100	0
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. cenocapsacia</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	0	0	100	0
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. anthina</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	0	0	0	0
<i>B. multivorans</i> (<i>n</i> = 1)	0	0	0	0
<i>B. cepacia</i> (<i>n</i> = 5)	20	0	100	20
<i>B. caribensis</i> DD <i>B. hospita</i> (<i>n</i> = 1)	0	0	100	0

* Varying results after 48 h of incubation in comparison to 24 h of incubation

Table 4. Mean values (± standard deviations) of fatty acid methyl ester groups

Species/species groups	12:00 (%)	14:01 (%)	14:00 (%)	15:00 (%)
<i>B. multivorans</i> DD <i>B. caryophili</i> (<i>n</i> = 24)	0 (0)	0.1 (0.1)	1.6 (1.1)	0.1 (0.1)
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> (<i>n</i> = 2)	4.4 (5.5)	0 (0)	0.6 (0.1)	0.3 (0)
<i>B. cepacia</i> DD <i>B. cenocapsacia</i> (<i>n</i> = 2)	0 (0)	0 (0)	2.2 (0.3)	0 (0)
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. cenocapsacia</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	0	0	2.7	0.3
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. anthina</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	1.2	0	0.7	0.2
<i>B. multivorans</i> (<i>n</i> = 1)	0	0	2.0	0
<i>B. glumae</i> (<i>n</i> = 1)	0	0	2.9	0.1
<i>B. cepacia</i> (<i>n</i> = 5)	0 (0)	0 (0)	1.2 (0.7)	0 (0)
<i>B. caribensis</i> DD <i>B. hospita</i> (<i>n</i> = 1)	0	0	2.1	0
Species/species groups	3OH-14:0	16:1*	16:01	16:1+
<i>B. multivorans</i> DD <i>B. caryophili</i> (<i>n</i> = 24)	2.1 (0.6)	0.1 (0.3)	12.5 (2.9)	0.4 (0.4)
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> (<i>n</i> = 2)	2.6 (1.2)	0 (0)	10.9 (0.6)	0.1 (0.14)
<i>B. cepacia</i> DD <i>B. cenocapsacia</i> (<i>n</i> = 2)	3.2 (0.4)	0 (0)	11.7 (0.4)	0.1 (0.1)
<i>B. cepacia</i> DD <i>B. vietnamiensis</i> DD <i>B. cenocapsacia</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	2.7	0	15.5	0.2

Table 4. (cont'd)

Species/species groups	3OH-14:0	16:1*	16:0:1	16:1+
<i>B. cepacia</i> DD <i>B. vietnamensis</i> DD <i>B. anthina</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	1.6	0	9.1	0
<i>B. multivorans</i> (<i>n</i> = 1)	2.7	0	10.2	0.3
<i>B. glumae</i> (<i>n</i> = 1)	2.6	0	12.0	0.5
<i>B. cepacia</i> (<i>n</i> = 5)	2.64 (0.4)	0 (0)	12.5 (2.6)	0.2 (0.2)
<i>B. caribensis</i> DD <i>B. hospita</i> (<i>n</i> = 1)	0.9	0	6.0	0
Species/species groups	16:0:0	17:0 Cyclo	17:0:0	18:1*
<i>B. multivorans</i> DD <i>B. caryophili</i> (<i>n</i> = 24)	26.8 (3.6)	15.1 (7.7)	0.4 (0.3)	1.6 (7.6)
<i>B. cepacia</i> DD <i>B. vietnamensis</i> (<i>n</i> = 2)	23.2 (1.3)	19.9 (0.3)	1.2 (0.35)	0 (0)
<i>B. cepacia</i> DD <i>B. cenocepacia</i> (<i>n</i> = 2)	24.6 (2.7)	14.8 (2.55)	0.6 (0)	0 (0)
<i>B. cepacia</i> DD <i>B. vietnamensis</i> DD <i>B. cenocepacia</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	27.1	11.8	1.1	0
<i>B. cepacia</i> DD <i>B. vietnamensis</i> DD <i>B. anthina</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	23.7	23.3	0.3	0
<i>B. multivorans</i> (<i>n</i> = 1)	26.8	23.8	0	0
<i>B. glumae</i> (<i>n</i> = 1)	27.3	10.5	0.5	0
<i>B. cepacia</i> (<i>n</i> = 5)	25.3 (0.8)	16.4 (3.77)	0.66 (0.2)	0 (0)
<i>B. caribensis</i> DD <i>B. hospita</i> (<i>n</i> = 1)	29.8	15.3	0.7	0
Species/species groups	18:0:1	18:1+	18:0:0	19:0 Cyclo
<i>B. multivorans</i> DD <i>B. caryophili</i> (<i>n</i> = 24)	27.85 (11.5)	0.2 (0.5)	1.8 (1.6)	9.5 (3.7)
<i>B. cepacia</i> DD <i>B. vietnamensis</i> (<i>n</i> = 2)	30.75 (2.5)	0 (0)	1.1 (0)	8.6 (0.71)
<i>B. cepacia</i> DD <i>B. cenocepacia</i> (<i>n</i> = 2)	31.1 (4.3)	0.35 (0.1)	1.8 (0.1)	9.8 (0.4)
<i>B. cepacia</i> DD <i>B. vietnamensis</i> DD <i>B. cenocepacia</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	31.3	0.3	2.2	4.8
<i>B. cepacia</i> DD <i>B. vietnamensis</i> DD <i>B. anthina</i> DD <i>B. ambifaria</i> (<i>n</i> = 1)	28.6	0	1.0	10.3
<i>B. multivorans</i> (<i>n</i> = 1)	21.0	0	0.5	12.9
<i>B. glumae</i> (<i>n</i> = 1)	37.4	1.0	1.0	4.5
<i>B. cepacia</i> (<i>n</i> = 5)	31.3 (1.7)	0.22 (0.3)	1.78 (0.5)	7.9 (2.9)
<i>B. caribensis</i> DD <i>B. hospita</i> (<i>n</i> = 1)	36.0	0	2.2	7.1

* Left-shifted peak
+ Right-shifted peak

between the *B. multivorans* DD *B. caryophili* cluster and the *B. cepacia* cluster makes an assignment of distinguishable species to these clusters nevertheless plausible.

The study has a number of limitations. First of all, not all strains could be included in all assessments. Even API 20 NE data of one isolate got lost. Some assessments could not be performed because the respective strains had died during passage from one culture or storage medium to another.

The more important limitation is the fact that no definite species assignments, e.g., by *recA* gene [14–16], *fur* gene [17], or *hisA* gene [18] sequence typing, or by multiple-locus sequence typing (MLST) [16, 19], could be performed. Limited financial capacities allowed for sequence analysis of the *rpsU* gene with a respective cluster assignment only. Accordingly, conclusions about phenotypic properties of *Burkholderia* spp. apart from the performed assignment on *rpsU* gene sequence level cannot be drawn from the presented data.

Conclusions

The assessed data demonstrate a broad phenotypic heterogeneity of clinical *Burkholderia* spp. isolates that are assigned to common *rpsU* gene sequence clusters. These data confirm previous results with sequences of reference strains [3] and underline the low discriminatory potential of *rpsU* gene sequencing of *Burkholderia* spp. isolates for diagnostic purposes, that is basically restricted to a delineation from the *rpsU-II* cluster comprising the *B. pseudomallei* complex [2, 3]. If any, the diagnostic use of *rpsU* gene sequence analysis could be limited to implementations into MLST schemes. Future studies are, however, necessary to confirm or deny this hypothesis.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Supporting information 1. Obtained *rpsU* gene sequences (fasta format) of the isolates. The species / species group assignments were based on published sequences of reference strains [3] and NCBI BLAST search. The length of ≤169 base pairs between the primers did not allow deposition at NCBI GenBank.

New <i>rpsU</i> sequences as obtained with the primers <i>fup1</i> and <i>fup2</i> (fasta format).
>B46_Burkholderia_multivorans_DD_Burkholderia_caryophylli CAGGCCTTGACGGCTGCTGCCTTCTTGCCTTGCCTGCGACTGCGGTGGCTTTCTGCTAG GACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTCGATAGCGCGACGAAAGC GGCGAATCGCCACTTCGAACGGCTCGTTCTTCAAAAGAACGTCGCGCCATA
>B51_Burkholderia_multivorans_DD_Burkholderia_caryophylli GGCTGCGCAGACGCTTGTGCAGGGCTTGACGGCTGCTGCCTTCTTGCCTGCGACTGCGAC TGCCTGCGCTTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTC CGATAGCGCGACGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGA ATCGTCGTCATA
>B52_Burkholderia_multivorans_DD_Burkholderia_caryophylli GCAGGCCTTGACGGCTGCTGCCTTCTTGCCTTGCCTGCGACTGCGGTGGCTTTCTGTA GGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTCGATAGCGCGACGAAAG CGGCCAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAACGTCGCGCCATA
>B53_Burkholderia_multivorans_DD_Burkholderia_caryophylli GCTGCGCAGACGCTTGTGCAGGGCTTGACGGCTGCTGCCTTCTTGCCTGCGACTGCGAC GCGGTGGCTTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTC GATAGCGCGACGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAA TCGCCGTCA
>B106_Burkholderia_multivorans_DD_Burkholderia_caryophylli CGTGGCTGCGCAGACGCTTGTGCAGGGCTTGACGGCTGCTGCCTTCTTGCCTGCG GAUTGCCTGCGCTTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATT TTTCGATAGCGCGACGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAA AAGAACGTCGTCATA
>B111_Burkholderia_multivorans_DD_Burkholderia_caryophylli TTGTGCAGGGCTTGACGGCTGCTGCCTTCTTGCCTGCGACTGCGGTGGCTTT CGTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTCGATAGCGCGACG AAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAACGTCGTCATA
>B120_Burkholderia_multivorans_DD_Burkholderia_caryophylli CAGACGCTTGTGCAGGGCTTGACGGCTGCTGCCTTCTTGCCTGCGACTGCGGT GGCTTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTCGATAGC GCGACGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAACGTC

GGTCGCGCAGACGCTTGTGCAGGCGCTTGACGGCTGCTGCCCTTGCCTGCCTGCGAC TGCCTGCGCTTTCTGACTGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTTT CGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGA ATCGTCGTATA
>P267_Burkholderia_multivorans_DD_Burkholderia_caryophylli GCTTGTGCAGGCGCTTGACGGCTGCTGCCCTTGCCTGCCTGCGACTGCCTGCGCTT TTCGTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTTTCTGATAGCGCGAC GAAAGCGGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAATCGTCGTATA
>P268_Burkholderia_multivorans_DD_Burkholderia_caryophylli CTTCTTGCCTGCGACTGCGCTGGCTTCTGCTAGGACTGGCGCTCGCGCAGTTCA GCGATCAGGCCATTTTTCTGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACG GCTCGTTCTTCAAAAGAATCGTCGTATA
>P269_Burkholderia_multivorans_DD_Burkholderia_caryophylli CATCTGGCTGCGCAGACGCTTGTGCAGGCGCTTGACGGCTGCTGCCCTTGCCTGCGCTT CGGACTGCGGTGGCTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCAT TTTTTCTGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTCTTCA AAAAGAATCGTCGTATA
>P270_Burkholderia_multivorans_DD_Burkholderia_caryophylli CTGCGCAGGCGCTTGTGCAGACGCTTGCAGACGCTTGACGGCTGCTGCCCTTGCCTGCGCTT CGGCTGGCTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTTTCTG ATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAAT CGTCGTATA
>P271_Burkholderia_multivorans_DD_Burkholderia_caryophylli GGCTGCGCAGGCGCTTGTGCAGACGCTTGCAGACGCTTGACGGCTGCTGCCCTTGCCTGCGCTT TGCCTGCGCTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTTT CGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGA ATCGTCGTATA
>P272_Burkholderia_multivorans_DD_Burkholderia_caryophylli CATCTGGCTGCGCAGACGCTTGTGCAGGCGCTTGACGGCTGCTGCCCTTGCCTGCGCTT CGGACTGCGGTGGCTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCAT TTTTTCTGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTCTTCA AAAAGAATCGTCGTATA
>P273_Burkholderia_multivorans_DD_Burkholderia_caryophylli GGCTGCGCAGACGCTTGTGCAGGCGCTTGACGGCTGCTGCCCTTGCCTGCGCTTGCCTGCGAC TGCCTGCGCTTCTGCTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTTT CGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGA ATCGTCGTATA

>P276_Burkholderia_multivorans_DD_Burkholderia_caryophylli
GACGGCTTGCCTTGCCTGCGACTGCGCTGGCTTCGTAGGACTGGCG TCGCGCAGTCAGCGATCAGGCCATTTCGATAGCGCGACGAAAGCGCGAATCG CCACTTCGAACGGCTCGTTCTTCAAAAGAACATCGTCGTCA
>P277_Burkholderia_multivorans_DD_Burkholderia_caryophylli
GGCTGCGCAGACGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTGCCTGCGCTTGC TGCCTCGTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATT CGATAGCGCGACGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAACATCGTCGTCA
>P320_Burkholderia_multivorans_DD_Burkholderia_caryophylli
GTGCAGGCGCTTGACGGCTGCTGCCTTGCCTGCGACTGCGCTGGCTTC TAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATTTCGATAGCGCGACGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAACATCGTCGTCA
>B54_Burkholderia_cepacia
GCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTGCCTGCGCTTGC CGGGCTGGCTTCGTAGGACTGGCGCTCACGCGATCAGGCCATT GATAGCACGGCGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAACATCGTCGTCA
>P258_Burkholderia_cepacia
GGCTGCGCAGGCGCTTATGCAGGCGCTTGACGGCTGCTGCCTTGCCTGCGCTTGC TGCCTCGTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATT CGATAGCACGGCGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAAAAGAACATCGTCGTCA
>P278_Burkholderia_cepacia
CTGGCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTGCCTGCGCT GACTGCGCTGGCTTCGTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATT TTTCGATAGCACGGCGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAA AAGAACATCGTCGTCA
>P308_Burkholderia_cepacia
GGCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTGCCTGCGCT CTGCGCTGGCTTCGTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCCATT TCGATAGCACGGCGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCAA AATCGTCGTCA
>P310_Burkholderia_cepacia
GCATCTGGCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTGCCT GCGGACTGCGCTGGCTTCGTAGGACTGGCGCTCGCGCAGTCAGCGATCAGGCC ATTTCGATAGCACGGCGAAAGCGCGAATGCCACTTCGAACGGCTCGTTCTTCA

TCAAAAGAATCGTCGTATA
>P8_Burkholderia_vietnamiensis_DD_Burkholderia_cepacia CTGGCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTCTGCGTTGCGG ACTGCGGTCGGCTTTCGTAGGACTGGCGTCGCGCAGCTCAGCGATCAGGCCATT TTTCGATAGCACGGCGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTTCTTCAA AGAATCGTCGTATA
>P154_Burkholderia_vietnamiensis_DD_Burkholderia_cepacia GCATCTGGCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTCTGCGTT GCGGACTGCGGTCGGCTTTCGTAGGACTGGCGTCGCGCAGCTCAGCGATCAGGCC ATTTTTTCGATAGCACGGCGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTTCTT TCAAAAGAATCGTCGTATA
>P112_Burkholderia_cepacia_DD_Burkholderia_cenocepacia CTGGCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTCTGCGCTTGCG GAUTGCGGTCGGCTTTCGTAAGACTGGCGCTCACGCAGTTCAGCGATCAGGCCATT TTTCGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTTCTTCAA AGAATCGTCGTATA
>P275_Burkholderia_cepacia_DD_Burkholderia_cenocepacia GGCTGCGCAGGCGCTTGTGCAGGCGCTTGACGGCTGCTGCCTTCTGCGCTTGCGGA CTGCGGTCGGCTTTCGTAAGACTGGCGCTCACGCAGTTCAGCGATCAGGCCATT TCGATAGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTTCTTCAAAG AATCGTCGTATA
>P113_Burkholderia_cepacia_DD_Burkholderia_vietnamiensis_DD_Burkholderia_ambifaria _DD_Burkholderia_cenocepacia CGAGCCGTTCGAAGTGGCGATTGCCGCTTCCGCTGCTATCGAAAAAAATGGCCTG ATCGCTGAAC TGCGCGAACGCCAGTCCTACGAAAAGCCGACCGCAGTCCGCAAGCGCA AGAAGGCAGCAGCCGTCAAGCGCCTGCATAAGCGCCTGCGCAGCCAGATGCTGCCGA AGAAGCTCCACA
>P153_Burkholderia_multivorans AGCCGTTCGAAGTGGCGATTGCCGCTTCCGCTGCGCTATCGAAAAAAATGGCCTGATC GCTGAAC TGCGCGAGGCCAGTCCTACGAAAAGCCGACCGCAGTCCGCAAGCGCAAG AAGGCAGCAGCCGTCAAGCGCCTGCACAAGCGTCTGCGCAGCCAGATGCTGCCGAAG AAGCTCCACA
>P259_Burkholderia_multivorans GCGCAGGCGCTTGTGCAGACGCTTGACGGCTGCTGCCTTCTGCGCTTGCGGACTGCG GTCGGCTTTCGTAGGACTGGCGCTCGCGCAGTTCAGCGATCAGGCCATT AGCGCGACGAAAGCGGCGAATGCCACTTCGAACGGCTCGTTTCTTCAAAGAATCG TCGTCATA

Supporting information 2. Measured minimum inhibitory concentrations (MIC) via broth microdilution.

(n=1)	0	0	0	0	0	0	0	0	0	0	1/2	0	0	0	0	0	0	0	1/2	0	0	0	0	0	0
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	1/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cenocepacia</i> (n=2)	0	0	0	0	0	0	0	0	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamiensis</i> DD																									
<i>B. cenocepacia</i> DD																									
<i>B. ambifaria</i> (n=1)																									
<i>B. multivorans</i> (n=1)	0	0	0	0	0	0	0	0	0	0	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cepacia</i> (n=5)	0	0	0	0	0	0	0	1/5	2/5	0	1/5	0	1/5	0	0	0	0	0	0	0	0	0	0	0	0
Trimethoprim																									
<i>B. multivorans</i> DD	0	0	0	0	0	4/2	9/2	3/2	2/2	0	3/2	0	1/2	0	1/2	0	1/2	0	0	0	0	0	0	0	0
<i>B. caryophili</i> (n=24)						4	4	4	4		4		4		4		4								
<i>B. cepacia</i> DD	0	0	0	0	0	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamiensis</i>																									
(n=1)																									
<i>B. cepacia</i> DD	0	0	0	0	0	1/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cenocepacia</i> (n=2)	0	0	0	0	0	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cepacia</i> DD	0	0	0	0	0	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamiensis</i> DD																									
<i>B. cenocepacia</i> DD																									
<i>B. ambifaria</i> (n=1)																									
<i>B. multivorans</i> (n=1)	0	0	0	0	0	0	0	0	0	0	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cepacia</i> (n=5)	0	0	0	0	0	0	3/5	1/5	1/5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fosfomycin																									
<i>B. multivorans</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. caryophili</i> (n=24)																							24/24	0	0
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamiensis</i>																									
(n=1)																									
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cenocepacia</i> (n=2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamiensis</i> DD																							1/1	0	0
<i>B. cenocepacia</i> DD																									
<i>B. ambifaria</i> (n=1)																									
<i>B. multivorans</i> (n=1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. cepacia</i> (n=5)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vancomycin																									
<i>B. multivorans</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1/2	0	0	0	0	0	0	0	0
<i>B. caryophili</i> (n=24)																	4								
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. vietnamiensis</i>																		1/1	0	0	0	0	0	0	

<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1/1	0	0	0	0	0	0
<i>B. vietnamiensis</i> (n=1)																							
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2/2	0	0	0	0	0	0
<i>B. cenocepacia</i> (n=2)																							
<i>B. cepacia</i> DD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1/1	0	0	0	0	0	0
<i>B. vietnamiensis</i> DD																							
<i>B. cenocepacia</i> DD																							
<i>B. ambifaria</i> (n=1)																							
<i>B. multivorans</i> (n=1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1/1	0	0	0	0	0	0
<i>B. cepacia</i> (n=5)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5/5	0	0	0	0	0	0