

Use of Comparative Genomics To Resolve an Unusual Case of Aminoglycoside Susceptibility in the Melioidosis Pathogen *Burkholderia pseudomallei* in Bangladesh

Kaestli, Mirjam; Farook, Saika; Jilani, Md Shariful Alam; Anwar, Shaheda; Siddiqui, Tanvir Ahmed; Mayo, Mark; Podin, Yuwana; Webb, Jessica R.; Dance, David A.B.; Currie, Bart J.

Published in:
The American journal of tropical medicine and hygiene

DOI:
[10.4269/ajtmh.24-0144](https://doi.org/10.4269/ajtmh.24-0144)

Published: 06/11/2024

Document Version
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):

Kaestli, M., Farook, S., Jilani, M. S. A., Anwar, S., Siddiqui, T. A., Mayo, M., Podin, Y., Webb, J. R., Dance, D. A. B., & Currie, B. J. (2024). Use of Comparative Genomics To Resolve an Unusual Case of Aminoglycoside Susceptibility in the Melioidosis Pathogen *Burkholderia pseudomallei* in Bangladesh. *The American journal of tropical medicine and hygiene*, 111(5), 1056-1059. <https://doi.org/10.4269/ajtmh.24-0144>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Using comparative genomics to resolve an unusual case of aminoglycoside susceptibility in the melioidosis pathogen *Burkholderia pseudomallei* in Bangladesh

Journal:	<i>American Journal of Tropical Medicine & Hygiene</i>
Manuscript ID	AJTMH-24-0144.R1
Manuscript Type:	Short Report
Date Submitted by the Author:	13-May-2024
Complete List of Authors:	<p>Kaestli, Mirjam; Menzies School of Health Research, Emerging and Infectious Diseases Farook, Saika; Ibrahim Medical College, Department of Microbiology Jilani, Md. Shariful Alam; Ibrahim Medical College, Department of Microbiology Anwar, Shaheda; Bangabandhu Sheikh Mujib Medical University, Department of Microbiology and Immunology Siddiqui, Tanvir Ahmed; Bangabandhu Sheikh Mujib Medical University, Department of Microbiology and Immunology Mayo, Mark; Menzies School of Health Research, Emerging and Infectious Diseases Podin, Yuwana; Universiti Malaysia Sarawak, Institute of Health and Community Medicine Webb, Jessica; Menzies School of Health Research, Global and tropical health; The University of Melbourne, Department of Microbiology and Immunology at The Peter Doherty Institute for Infection and Immunity Dance, David; Laos-Oxford-Mahosot-Wellcome Research Unit, Microbiology Laboratory; London School of Hygiene & Tropical Medicine, Faculty of Infectious and Tropical Diseases Currie, Bart; Menzies School of Health Research, Global and tropical health; Royal Darwin Hospital, Infectious Diseases Department</p>
Key Words:	Melioidosis, Bacterial Diseases, Drug Resistance, Emerging Diseases, Genomics

1 **Using comparative genomics to resolve an unusual case of aminoglycoside**
2 **susceptibility in the melioidosis pathogen *Burkholderia pseudomallei* in**
3 **Bangladesh**

4

5 Kaestli, Mirjam¹; Farook, Saika²; Jilani, Md. Shariful Alam²; Anwar, Shaheda³;
6 Siddiqui, Tanvir Ahmed³; Mayo, Mark¹; Podin, Yuwana⁴; Webb, Jessica R^{1,5}; Dance,
7 David AB^{6,7}; Currie, Bart J^{1,8*}

8

9 1 Menzies School of Health Research, Charles Darwin University, Darwin, Northern
10 Territory, Australia; mirjam.kaestli@menzies.edu.au; mark.mayo@menzies.edu.au;
11 bart.currie@menzies.edu.au

12 2 Department of Microbiology, Ibrahim Medical College, Dhaka, Bangladesh;
13 sairana15@yahoo.com; jilanimsa@gmail.com;

14 3 Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical
15 University, Dhaka, Bangladesh; shahedaanwar17@gmail.com;
16 shaon_mbo@yahoo.com

17 4 Institute of Health and Community Medicine, Universiti Malaysia Sarawak,
18 Sarawak, Malaysia; pyuwana@unimas.my

19 5 Department of Microbiology and Immunology at The Peter Doherty Institute for
20 Infection and Immunity, The University of Melbourne, Melbourne, Victoria, Australia
21 jessica.webb@unimelb.edu.au

22 6 Lao-Oxford-Mahosot Hospital-Wellcome Trust Unit, Microbiology Laboratory,
23 Mahosot Hospital, Vientiane, Lao People's Democratic Republic
24 David.d@tropmedres.ac

25 7 Faculty of Infectious and Tropical Diseases, London School of Hygiene and
26 Tropical Medicine, London, UK

27 8 Infectious Diseases Department, Royal Darwin Hospital, Darwin, Northern Territory
28 Australia

29

30 * Menzies School of Health Research, PO Box 41096, Casuarina NT 0811, Australia

31 Phone: +61 8 8946 8600; Email: bart.currie@menzies.edu.au

32

33 Running head: genomics of aminoglycoside susceptible *Burkholderia pseudomallei*

34

35 Key words: Melioidosis, *Burkholderia pseudomallei*, Bangladesh, whole genome
36 sequencing, aminoglycoside susceptibility

37

38 Word count abstract: 149

39 Word count text: 1,334

40 Number of figures: 1

41 Number of tables: 1

42 Supplementary material: Supplement Table 1, Supplement Figure 1

43

44

45 **Abstract**

46

47 Melioidosis is an emerging tropical infectious disease with a rising global burden
48 caused by the environmental bacterium *Burkholderia pseudomallei*. It is endemic in
49 Southeast and South Asia, including Bangladesh. A rare aminoglycoside-susceptible
50 *B. pseudomallei* isolate (Y2019) has recently been reported from a melioidosis
51 patient in Dhaka, Bangladesh. To understand the geographical origins of Y2019 we
52 whole genome sequenced it and ten other isolates from Bangladesh. In a
53 phylogenetic tree with a global set of *B. pseudomallei* genomes, most Bangladeshi
54 genomes clustered tightly within the Asian clade. In contrast, Y2019 was closely
55 related to ST881 isolates from Sarawak, Malaysian Borneo, a gentamicin-sensitive
56 sequence type, suggesting infection in Borneo. Y2019 also contained the same
57 gentamicin sensitivity conferring nonsynonymous mutation in the drug efflux pump
58 encoding *amrB* gene. In the absence of a full travel history, whole genome
59 sequencing and bioinformatics tools have revealed the likely origin of this rare
60 isolate.

61

62

63

64 Melioidosis is an often severe infectious disease caused by the opportunistic
65 pathogen *Burkholderia pseudomallei*, a soil- and water-dwelling sapronotic
66 bacterium ¹. It is endemic in southern Asia and northern Australia and emerging in
67 the tropical belt worldwide, with a rising global burden ^{1, 2}. It is a neglected and often
68 misdiagnosed disease, with farmers in rural settings of lower- and middle-income
69 countries being at highest risk, particularly those with co-morbidities such as
70 diabetes mellitus ².

71 Melioidosis is endemic but significantly underdiagnosed in Bangladesh, a highly
72 populous and agricultural country in South Asia with a growing diabetes burden ^{3, 4}. It
73 has been detected sporadically over recent decades since the earliest case in
74 Bangladesh (known as East Pakistan at that time) was described in 1960 in a British
75 sailor whose ship was deposited in a rice paddy field by a cyclone ³. A systematic
76 study of *B. pseudomallei* occurrence in Bangladesh recovered the bacterium from
77 soil and found a seropositivity rate of above 20% in the population of three districts
78 where melioidosis cases have been recorded ⁵. Multi-locus sequence typing (MLST,
79 <https://pubmlst.org/organisms/burkholderia-pseudomallei>) of 22 mainly clinical *B.*
80 *pseudomallei* isolates revealed both common sequence types (STs) which had also
81 been reported from Southeast Asian countries, and novel STs only described from
82 Bangladesh ⁶, confirming its endemic status for melioidosis.
83 *B. pseudomallei* is intrinsically resistant to a wide range of antimicrobial agents
84 including macrolides and aminoglycosides to which resistance is mediated by the
85 multidrug efflux system AmrAB-OprA. The aminoglycoside gentamicin is used in the
86 selective culture medium (Ashdown's) for *B. pseudomallei* isolation from clinical and
87 environmental specimens ^{7, 8}. *B. pseudomallei* isolates susceptible to gentamicin
88 have only rarely been reported, partly because they may be missed when using such

89 culture media ^{9, 10, 11}. A gentamicin-sensitive clinical strain (708a – ST23) was
90 identified in 1998 in Thailand ¹¹. Its genome had a 131 kb deletion including the
91 *amrAB* operon ¹². The gentamicin susceptibility of a strain identified in 2023 in India
92 (ST1124) was due to an insertion of two nucleotides into *amrB* resulting in a
93 frameshift mutation ¹⁰. Sarawak (Malaysia) on Borneo Island has a high incidence of
94 gentamicin-sensitive *B. pseudomallei* infections due to the expansion of specific
95 sequence types, ST881 and its single-locus variant ST997, which carry a
96 nonsynonymous mutation within *amrB* ¹³. These isolates also have a synonymous
97 single nucleotide polymorphism (SNP) and 11-nucleotide insertion in the *piuA* gene
98 which encodes an outer membrane receptor, resulting in a frameshift mutation and *in*
99 *vitro* non-susceptibility to cefiderocol ¹⁴. This siderophore cephalosporin antibiotic
100 was designed for difficult-to-treat β -lactam-resistant and multidrug resistant (MDR)
101 Gram-negative bacteria. *B. pseudomallei* isolates are usually highly susceptible to
102 cefiderocol ¹⁴.

103 A gentamicin-sensitive *B. pseudomallei* (Y2019) infection was reported from
104 Bangladesh in 2019 ¹⁵. The isolate was from a 55-year-old Bengali, non-diabetic
105 male admitted to the Dhaka Medical College Hospital with a history of high-grade
106 fever, cough and weight loss. Culture of pus from an elbow joint aspirate grew
107 bacteria on Blood and MacConkey agar which were identified as *B. pseudomallei* by
108 biochemical, PCR and monoclonal antibody latex agglutination tests. No growth was
109 detected on modified Ashdown's selective media containing gentamicin, and
110 aminoglycoside susceptibility was confirmed by antimicrobial susceptibility testing.
111 He recovered with standard treatment for melioidosis ¹⁵.

112 We sequenced the whole genome of Y2019 together with ten isolates (8 clinical
113 and 2 environmental) from Bangladesh which were recently subjected to MLST

114 analyses ⁶ (Table 1). This was to assess whether the gentamicin susceptibility was
 115 due to a known mutation in the *amrAB-OprA* operon and to analyse the relatedness
 116 of Y2019 to the other Bangladeshi isolates and to a global set of publicly available *B.*
 117 *pseudomallei* genomes.

118

119 **Table 1:** Collection year, location in Bangladesh and source of *Burkholderia*
 120 *pseudomallei* isolates which were whole genome sequenced in this study (“Env”
 121 environmental) ⁶ See Supplement Figure 1 for a map of locations.

Isolate	Year	Location	Source	<i>in silico</i> MLST
Y2019	2019	Dhaka	Human	881
CS5414	2015	Tangail	Human	756
CS89	2015	Tangail	Human	1007
CS2317	2009	Tangail	Human	756
CS6260	2015	Mymensingh	Human	56
CS90	2014	Mymensingh	Human	1007
CS60	2015	Gazipur	Human	486
K23	2012	Gazipur	Env	1005
K35	2012	Gazipur	Env	1005
NB	2010	Gazipur	Human	1005
CS4987	2015	Cumilla	Human	188

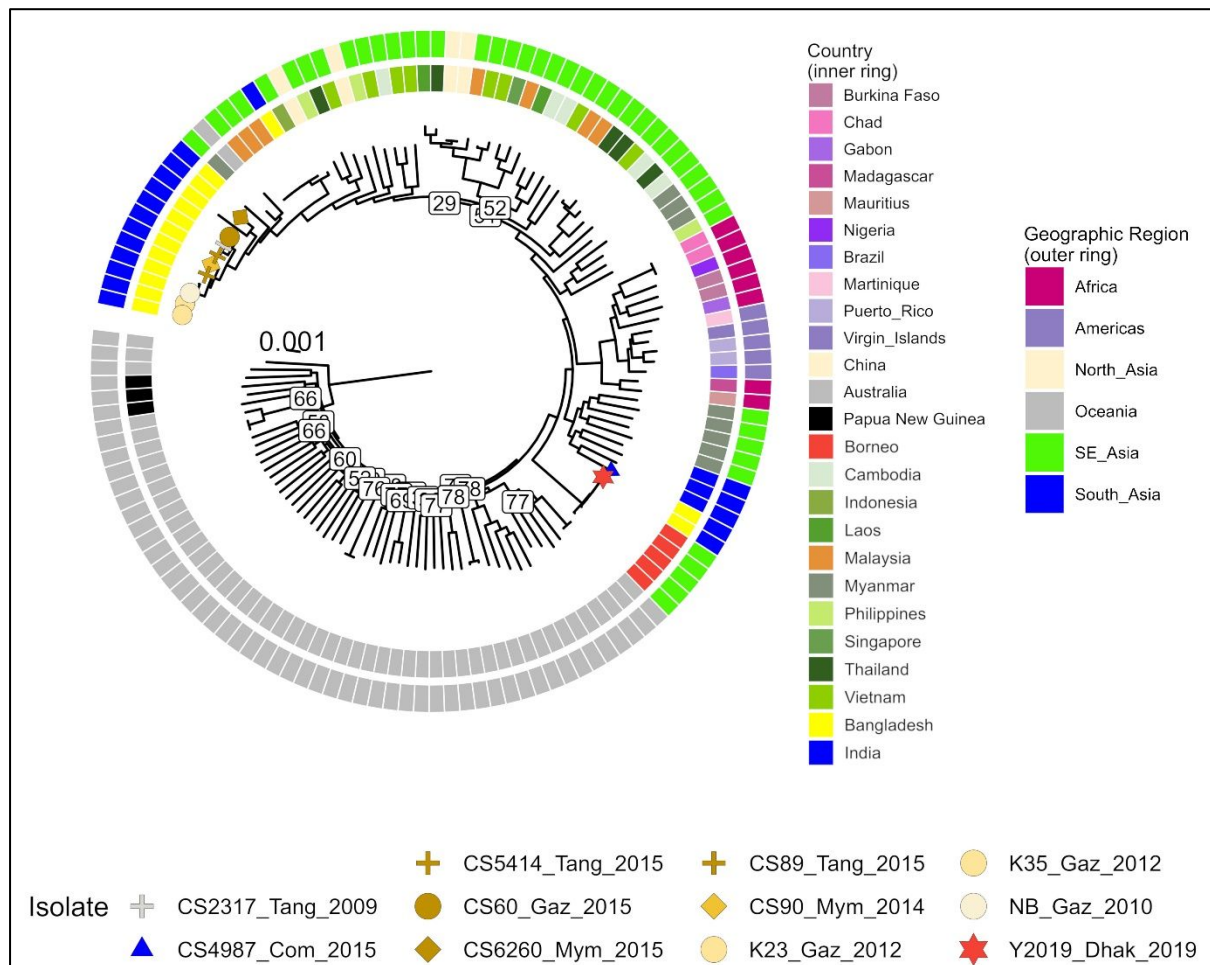
122

123 Sample collection, *B. pseudomallei* culture and DNA extraction of eleven
 124 isolates were as previously described ⁶. Whole genome sequencing, phylogenetic
 125 analysis and *in-silico* MLST assignment were performed as previously described ¹⁶.
 126 The eleven *B. pseudomallei* genomes have been deposited in the NCBI Sequence
 127 Read Archive (SRA) under BioProject accession number PRJNA1077088. *De novo*
 128 genome assemblies of Illumina paired-end reads were generated using Shovill
 129 (default settings including the use of SPADes – minimum length 1,000 bp)
 130 (<https://github.com/tseemann/shovill>). To explore mutations in the *amrB* and *piuA*

131 genes, BLAST analyses were used (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>)
132 (BP1026B_II1275; Burkholderia Genome Database (www.burkholderia.com) as *piuA*
133 reference ¹⁴) and the Antimicrobial Resistance Detection and Prediction tool ARDaP
134 ¹⁷.

135 Phylogenetic analysis showed a distinct clustering of nine of the eleven newly
136 sequenced Bangladeshi isolates within the Southeast Asian clade (Figure 1). The
137 environmental isolates K23 and K35 were identical with no core SNPs between
138 them. They were from soil samples collected in Gazipur in 2012 and were most
139 related to a clinical isolate from Gazipur collected two years earlier, with 710 core
140 SNP differences and the same ST1005. The nine isolates consisted of five STs and
141 originated from four locations across Bangladesh 100-200 km apart (Table 1).
142 Compared to the high genetic diversity across Asian *B. pseudomallei* genomes, the
143 diversity of these nine genomes was notably limited, with short branches and less
144 than 6,500 core SNPs between them. They also clustered with three of the four
145 additional publicly available *B. pseudomallei* genomes from Bangladesh
146 (H034580128, H061220286 and H103520155 – see Supplement Table 1). While the
147 sampling locations were relatively close (less than 200 km apart)(Supplement Figure
148 1), a considerably higher *B. pseudomallei* diversity has been observed in similar
149 sized areas in other endemic regions ¹⁸. Given large rivers intersect Bangladesh,
150 water and floods likely play an important role in *B. pseudomallei* dissemination,
151 potentially also limiting the development of distinct geographical populations in
152 Bangladesh in the absence of major geographical barriers. With only a limited
153 number of isolates analysed in this study, more extensive environmental sampling,
154 isolation, and whole genome sequencing of *B. pseudomallei* from Bangladesh are
155 necessary to confirm the generalisability of this observed limited diversity.

156 In contrast to the distinct Bangladesh clade, isolate CS4987 clustered with
 157 three isolates from India, suggesting that the patient infected with CS4987 might
 158 have acquired melioidosis while visiting that neighbouring country (with CS4987
 159 having more than 13,000 SNPs difference to genomes of the Bangladesh cluster).
 160



161

162 **Figure 1:** Maximum likelihood phylogenetic tree based on 147,216 core SNPs of *B.*
 163 *pseudomallei* genomes from this study (n=11) and publicly available global genomes
 164 (n=125) (Supplement Table 1). The outer colour ring refers to the geographic region
 165 while the inner ring shows the country of origin. Borneo in the country legend refers
 166 to Malaysian Borneo (Sarawak). The scale bar indicates substitutions per site.
 167 Bootstrap support (based on 1,000 replicates) for branches below 80% are shown.
 168 The tree was rooted using MSHR0668 which was the most ancestral *B.*

169 *pseudomallei* strain in a global phylogenetic study of *B. pseudomallei* including
170 closely related *Burkholderia* species¹⁹. The isolate names include the sample
171 collection location in Bangladesh “Tang” Tangail, “Com” Cumilla, “Gaz” Gazipur,
172 “Mym” Mymensingh, “Dhak” Dhaka and year of collection.

173

174 The gentamicin-susceptible isolate Y2019, on the other hand, was closely
175 related to genomes from Sarawak, Malaysian Borneo, with only 20 to 400 SNPs
176 difference (as compared to 14,800 SNPs to K23 of the Bangladesh cluster). As with
177 the other genomes from Sarawak, Y2019 also belonged to ST881, the gentamicin-
178 sensitive ST, and it contained the same nonsynonymous mutation within *amrB*¹³.
179 Similarly, Y2019 also harboured the *piuA* gene mutations with one SNP and an
180 eleven nucleotide insertion as in the ST881 isolates from Sarawak¹⁴. These results
181 strongly suggest that Y2019 is linked to Sarawak. The patient had been noted to
182 have spent 10 years in Malaysia working in the construction industry in Malacca¹⁵.
183 We have been unsuccessful in finding the patient to ascertain if he also worked in
184 Sarawak. It is conceivable that ST881 may have been introduced to Malacca;
185 however, no reports on aminoglycoside susceptible *B. pseudomallei* from this region
186 were found and there were only 20 SNPs difference between Sarawak isolates and
187 Y2019. Therefore, it seems more likely that Y2019 was acquired in Sarawak. The
188 resource-rich state of Sarawak has been recruiting large numbers of migrant
189 workers, including from Bangladesh, to sustain its growing economy²⁰. There have
190 also been reports potentially linking melioidosis cases in Sarawak to the construction
191 of the large-scale Bakun dam²¹.

192 In conclusion, aminoglycoside susceptibility in *B. pseudomallei* is rarely
193 reported, likely due to its very rare occurrence globally, but with the additional issue

194 of lack of growth in standard selective media used for culture of *B. pseudomallei*. By
195 using comparative genomics, readily available bioinformatics tools and public
196 genomes and sequence type databases, we have unveiled the likely origin of an
197 unusual aminoglycoside-susceptible *B. pseudomallei* from a patient with melioidosis
198 diagnosed in Bangladesh. Our results support the suggestion that the patient was
199 likely infected in Sarawak, Malaysia, where gentamicin susceptible *B. pseudomallei*
200 of the same ST and with the same mutation within *amrB* have been commonly
201 found.

202

203 **Acknowledgements**

204 We would like to thank Vanessa Rigas and Kelly McCrory for laboratory support.

205

206 **Financial Support**

207

208

209

210 **References**

- 211 1. Meumann EM, Limmathurotsakul D, Dunachie SJ, Wiersinga WJ, Currie BJ, 2024.
212 *Burkholderia pseudomallei* and melioidosis. *Nature Reviews Microbiology* 22: 155-
213 169.
- 214 2. Birnie E, Virk HS, Savelkoel J, Spijker R, Bertherat E, Dance DAB, Limmathurotsakul D,
215 Devleeschauwer B, Haagsma JA, Wiersinga WJ, 2019. Global burden of melioidosis
216 in 2015: a systematic review and data synthesis. *The Lancet Infectious Diseases* 19:
217 892-902.
- 218 3. Chowdhury FR, Roy CK, Barai L, Paul S, Chowdhury FUH, Mazumder S, Farook S, Jilani
219 MSA, 2021. Melioidosis: A Neglected Infection in Bangladesh. *Journal of Medicine*
220 22: 139-145.
- 221 4. Haque M, Islam S, Kamal ZM, Akter F, Jahan I, Rahim MSA, Sultana N, Alam AM,
222 Munzur EM, Halim-Khan MA, Deeba F, Bakar MA, Nahar S, Mozaffor M, Urmi UL,
223 Saikat TR, Islam MZ, Haque M, Iqbal S, Hossain MM, Naher N, Allocati E, Godman B,
224 2021. Ongoing efforts to improve the management of patients with diabetes in
225 Bangladesh and the implications. *Hospital Practice* 49: 266-272.
- 226 5. Jilani MSA, Robayet JAM, Mohiuddin M, Hasan MR, Ahsan CR, Haq JA, 2016.
227 *Burkholderia pseudomallei*: Its Detection in Soil and Seroprevalence in Bangladesh.
228 *PLOS Neglected Tropical Diseases* 10: e0004301.
- 229 6. Jilani MSA, Farook S, Bhattacharjee A, Barai L, Ahsan CR, Haq JA, Tuanyok A, 2023.
230 Phylogeographic characterization of *Burkholderia pseudomallei* isolated from
231 Bangladesh. *PLOS Neglected Tropical Diseases* 17: e0011823.
- 232 7. Ashdown LR, 1979. An improved screening technique for isolation of *Pseudomonas*
233 *pseudomallei* from clinical specimens. *Pathology* 11: 293-297.
- 234 8. Limmathurotsakul D, Dance DA, Wuthiekanun V, Kaestli M, Mayo M, Warner J,
235 Wagner DM, Tuanyok A, Wertheim H, Cheng TY, 2013. Systematic review and
236 consensus guidelines for environmental sampling of *Burkholderia pseudomallei*.
237 *PLoS Negl Trop Dis* 7: e2105.
- 238 9. Corea EM, Merritt AJ, Ler YH, Thevanesam V, Inglis TJ, 2016. Sri Lankan National
239 Melioidosis Surveillance Program Uncovers a Nationwide Distribution of Invasive
240 Melioidosis. *Am J Trop Med Hyg* 94: 292-8.
- 241 10. Bakthavatchalam YD, Basu S, Shankar A, Ramaiah S, Anbarasu A, Veeraraghavan B,
242 2023. Genomics and structural insight into the masking of gentamicin-resistance in
243 clinical *Burkholderia pseudomallei* strain VB29710 from India. *Diagn Microbiol Infect*
244 *Dis* 105: 115878.
- 245 11. Harley VS, Dance DA, Drasar BS, Tovey G, 1998. Effects of *Burkholderia pseudomallei*
246 and other *Burkholderia* species on eukaryotic cells in tissue culture. *Microbios* 96:
247 71-93.
- 248 12. Trunck LA, Propst KL, Wuthiekanun V, Tuanyok A, Beckstrom-Sternberg SM,
249 Beckstrom-Sternberg JS, Peacock SJ, Keim P, Dow SW, Schweizer HP, 2009.
250 Molecular Basis of Rare Aminoglycoside Susceptibility and Pathogenesis of
251 *Burkholderia pseudomallei* Clinical Isolates from Thailand. *PLOS Neglected Tropical*
252 *Diseases* 3: e519.
- 253 13. Podin Y, Sarovich DS, Price EP, Kaestli M, Mayo M, Hii K, Ngian H, Wong S, Wong I,
254 Wong J, 2014. *Burkholderia pseudomallei* isolates from Sarawak, Malaysian Borneo,

- 255 are predominantly susceptible to aminoglycosides and macrolides. Antimicrobial
256 agents and chemotherapy 58: 162-166.
- 257 14. Hall CM, Somprasong N, Hagen JP, Nottingham R, Sahl JW, Webb JR, Mayo M, Currie
258 BJ, Podin Y, Wagner DM, Keim P, Schweizer HP, 2023. Exploring Cefiderocol
259 Resistance Mechanisms in *Burkholderia pseudomallei*. Antimicrobial Agents and
260 Chemotherapy 67: e00171-23.
- 261 15. Farook S, Jilani MSA, Akhter A, Haq J, 2020. Melioidosis by aminoglycoside
262 susceptible *Burkholderia pseudomallei*: First case in Bangladesh. IMC Journal of
263 Medical Science 14.
- 264 16. Guterres H, Gusmao C, Pinheiro M, Martins J, Odio G, Maia C, da Conceicao V, Soares
265 M, Osorio C, da Silva ES, Tilman A, Givney R, Oakley T, Yan J, Toto L, Amaral E, James
266 R, Buising K, Mayo M, Kaestli M, Webb JR, Baird RW, Currie BJ, Francis JR, Muhi S,
267 2023. Melioidosis in Timor-Leste: First Case Description and Phylogenetic Analysis.
268 Open Forum Infectious Diseases 10.
- 269 17. Madden DE, Webb JR, Steinig EJ, Currie BJ, Price EP, Sarovich DS, 2021. Taking the
270 next-gen step: Comprehensive antimicrobial resistance detection from *Burkholderia*
271 *pseudomallei*. EBioMedicine 63: 103152-103152.
- 272 18. Kaestli M, O'Donnell M, Rose A, Webb J, Mayo M, Currie B, Gibb K, 2019.
273 Opportunistic pathogens and large microbial diversity detected in source-to-
274 distribution drinking water of three remote communities in Northern Australia. PLoS
275 NTD In press.
- 276 19. Price EP, Currie BJ, Sarovich DS, 2017. Genomic Insights Into the Melioidosis
277 Pathogen, *Burkholderia pseudomallei*. Current Tropical Medicine Reports 4: 95-102.
- 278 20. Aeria A, 2016. Economic development via dam building: The role of the state
279 government in the sarawak corridor of renewable energy and the impact on
280 environment and local communities. Southeast Asian Studies 5.
- 281 21. Sia TLL, Mohan A, Ooi M-H, Chien S-L, Tan L-S, Goh C, Pang DCL, Currie BJ, Wong J-S,
282 Podin Y, 2021. Epidemiological and Clinical Characteristics of Melioidosis Caused by
283 Gentamicin-Susceptible *Burkholderia pseudomallei* in Sarawak, Malaysia. Open
284 Forum Infectious Diseases 8.
- 285

286

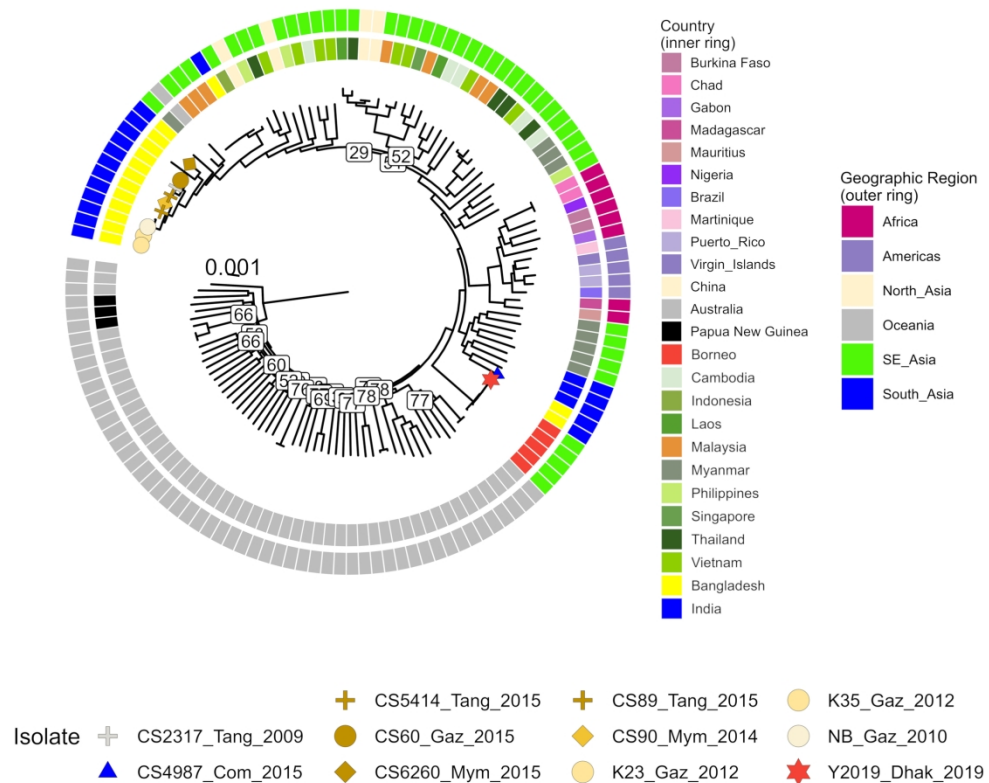


Figure 1

194x154mm (300 x 300 DPI)

Supplementary Table 1: Isolates in Phylogenetic Tree

Isolate	Year	MLST	Geographical Area	Country of Origin	Accession	Genome Reference (if applicable)
MSHR11369	2017	36	Oceania	Australia	SAMN12824619	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-141
MSHR10743	2017	109	Oceania	Australia	SAMN12824552	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-8
MSHR10130		113	Oceania	Australia	SRR9333951	Kaestli et al., PlosNTD 2019 doi.org/10.1371/journal.pntd.0007672
MSHR10167	2017	131	Oceania	Australia	SAMN12824497	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-19
MSHR10751	2017	132	Oceania	Australia	SAMN12824554	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-76
MSHR10246	2017	144	Oceania	Australia	SAMN12824504	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-26
MSHR1555	2003	239	Oceania	Australia	ERR539745	Chewapreecha et al 2017
MSHR11042	2017	279	Oceania	Australia	SAMN12824580	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-102
MSHR10166	2017	320	Oceania	Australia	SAMN12824496	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-18
MSHR6137		325	Oceania	Australia	AXDS00000000	McRobb et al., J Clin Microbiol 2015 53(4):1144-48
MSHR10888	2017	326	Oceania	Australia	SAMN12824566	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-88

MSHR10117	2017	327	Oceania	Australia	SAMN12824493	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-15
MSHR0091		331	Oceania	Australia	SRR8790694	Webb et al., PLOS NTD 2019 doi.org/10.1371/journal.pntd.0007369
MSHR10690	2017	335	Oceania	Australia	SAMN12824540	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-62
MSHR11384	2017	362	Oceania	Australia	SAMN12824620	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-142
MSHR9671	2016	456	Oceania	Australia	SAMN12824487	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-9
MSHR11277	2017	462	Oceania	Australia	SAMN12824609	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-131
MSHR10901	2017	464	Oceania	Australia	SAMN12824567	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-89
MSHR10622	2017	466	Oceania	Australia	SAMN12824531	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-53
MSHR10100	2017	472	Oceania	Australia	SAMN12824490	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-12
MSHR10269	2017	553	Oceania	Australia	SAMN12824507	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-29
MSHR5848		553	Oceania	Australia	CP008909,CP008910	Daligault et al., Genome Announc 2014 2(6):e01106-14
MSHR11290	2017	561	Oceania	Australia	SAMN12824611	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-133

MSHR10541	2017	562	Oceania	Australia	SAMN12824528	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-50
MSHR10978	2017	566	Oceania	Australia	SAMN12824574	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-96
MSHR10697	2017	616	Oceania	Australia	SAMN12824542	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-64
MSHR11267	2017	639	Oceania	Australia	SAMN12824607	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-129
MSHR10283		678	Oceania	Australia	SRR9333956	Kaestli et al., PlosNTD 2019 doi.org/10.1371/journal.pntd.0007672
MSHR10274		731	Oceania	Australia	SRR9333954	Kaestli et al., PlosNTD 2019 doi.org/10.1371/journal.pntd.0007672
MSHR9932		734	Oceania	Australia	SRR8790686	Webb et al., PLOS NTD 2019 doi.org/10.1371/journal.pntd.0007369
MSHR0938		737	Oceania	Australia	SRR8790683	Webb et al., PLOS NTD 2019 doi.org/10.1371/journal.pntd.0007369
MSHR2254		770	Oceania	Australia	SRR8790690	Webb et al., PLOS NTD 2019 doi.org/10.1371/journal.pntd.0007369
MSHR11194	2017	801	Oceania	Australia	SAMN12824596	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-118
MSHR11424		807	Oceania	Australia	SRR8790701	Webb et al., PLOS NTD 2019 doi.org/10.1371/journal.pntd.0007369
MSHR10550	2017	809	Oceania	Australia	SAMN12824529	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-51
MSHR10693	2017	813	Oceania	Australia	SAMN12824541	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-63

MSHR10746	2017	982	Oceania	Australia	SAMN12824553	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-75
MSHR10526	2017	984	Oceania	Australia	SAMN12824525	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-47
MSHR4378		1025	Oceania	Australia	JQDP00000000	Johnson et al., Genome Announc 2015 3(1):e01282-14
MSHR7343		1030	Oceania	Australia	JQDM00000000	Johnson et al., Genome Announc 2015 3(1):e01282-14
MSHR2618		1485	Oceania	Australia	SRR8790693	Webb et al., PLOS NTD 2019 doi.org/10.1371/journal.pntd.0007369
MSHR10126		1591	Oceania	Australia	SRR9333952	Kaestli et al., PlosNTD 2019 doi.org/10.1371/journal.pntd.0007672
MSHR10275		1651	Oceania	Australia	SRR9333953	Kaestli et al., PlosNTD 2019 doi.org/10.1371/journal.pntd.0007672
MSHR10259	2017	1654	Oceania	Australia	SAMN12824506	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-28
MSHR11266	2017	1655	Oceania	Australia	SAMN12824606	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-128
MSHR10355	2017	1656	Oceania	Australia	SAMN12824512	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-34
MSHR11335	2017	1658	Oceania	Australia	SAMN12824615	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-137
MSHR11255	2017	1659	Oceania	Australia	SAMN12824604	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-126

MSHR11092	2017	1660	Oceania	Australia	SAMN12824589	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-111
MSHR10619	2017	1704	Oceania	Australia	SAMN12824530	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-52
MSHR10635	2017	1705	Oceania	Australia	SAMN12824533	Rachlin et al., SciRep 2020 doi.org/10.1038/s41598-020-62300-55
MSHR668	1995	129	Oceania	Australia	SRR1617372	
6_CDC	1960	46	Asia	Bangladesh	ERR311033	Chewapreecha et al 2017
H061220286	2006	1007	Asia	Bangladesh	ERR298751	Chewapreecha et al 2017
H034580128	2003	unknown	Asia	Bangladesh	ERR298750	Chewapreecha et al 2017
H103520155	2010	unknown	Asia	Bangladesh	ERR298752	Chewapreecha et al 2017
H061740680	2006	92	Americas	Brazil	ERR298754	Chewapreecha et al 2017
MSHR7964		1121	Africa	Burkina Faso	SRR3145394	Sarovich et al mSphere 2016 1(2): e00089-15
MSHR7965		1122	Africa	Burkina Faso	SRR3145395	Sarovich et al mSphere 2016 1(2): e00089-15
E562	2006	494	Asia	Cambodia	ERR178253	Chewapreecha et al 2017
E569	2006	510	Asia	Cambodia	ERR178254	Chewapreecha et al 2017
SR_025	2007	690	Asia	Cambodia	ERR178251	Chewapreecha et al 2017
SR_020	2007	693	Asia	Cambodia	ERR178250	Chewapreecha et al 2017
SR_039	2008	694	Asia	Cambodia	ERR178245	Chewapreecha et al 2017
5691	1956	82	Africa	Chad	ERR298779	Chewapreecha et al 2017
MSHR6969	1956	82	Africa	Chad	ERR298347	Chewapreecha et al 2017
Hainan_106	1996	50	Asia	China	ERR298758	Chewapreecha et al 2017
E0702	1999	70	Asia	China	ERR539771	Chewapreecha et al 2017
HK2	1987	70	Asia	China	ERR539766	Chewapreecha et al 2017
Hainan1	1996	unknown	Asia	China	ERR298757	Chewapreecha et al 2017

Gabon_Bp	2013	1127	Africa	Gabon	ERR403716	Chewapreecha et al 2017
G9709	1995	43	Asia	India	ERR311037	Chewapreecha et al 2017
H054640145	2005	344	Asia	India	ERR298759	Chewapreecha et al 2017
H103360117	2010	unknown	Asia	India	ERR298760	Chewapreecha et al 2017
MSHR2056	2005	46	Asia	Indonesia	ERR539754	Chewapreecha et al 2017
MM37	2004	500	Asia	Laos	ERR162603	Chewapreecha et al 2017
MM39	2004	511	Asia	Laos	ERR162598	Chewapreecha et al 2017
MSHR7966		1043	Africa	Madagascar	SRR3145396	Sarovich et al mSphere 2016 1(2): e00089-15
LN_10	NA	46	Asia	Malaysia	ERR298768	Chewapreecha et al 2017
LN_31348	NA	46	Asia	Malaysia	ERR298765	Chewapreecha et al 2017
LN_6	NA	46	Asia	Malaysia	ERR298766	Chewapreecha et al 2017
LN_34170	1977	51	Asia	Malaysia	ERR298763	Chewapreecha et al 2017
MSHR0315	1994	232	Asia	Malaysia	ERR311047	Chewapreecha et al 2017
LN_29564	NA	289	Asia	Malaysia	ERR298764	Chewapreecha et al 2017
LN_22892	NA	438	Asia	Malaysia	ERR298767	Chewapreecha et al 2017
MSHR7400	2010	92	Americas	Martinique	ERR298359	Chewapreecha et al 2017
Green	2004	unknown	Africa	Mauritius	ERR311038	Chewapreecha et al 2017
MSHR12627	2017	56	Asia	Myanmar	SAMN15594681	Webb et al., Sci Rep, 2020
MSHR12629	2018	90	Asia	Myanmar	SAMN15594683	Webb et al., Sci Rep, 2020
MSHR12634	2018	346	Asia	Myanmar	SAMN15594687	Webb et al., Sci Rep, 2020
MSHR12633	2018	1371	Asia	Myanmar	SAMN15594686	Webb et al., Sci Rep, 2020
MSHR12636	2018	1752	Asia	Myanmar	SAMN15594688	Webb et al., Sci Rep, 2020
MSHR12637	2018	1753	Asia	Myanmar	SAMN15594689	Webb et al., Sci Rep, 2020
MSHR12833	2018	1765	Asia	Myanmar	SAMN15594694	Webb et al., Sci Rep, 2020
MSHR12837	2018	1766	Asia	Myanmar	SAMN15594695	Webb et al., Sci Rep, 2020
MSHR12647	2018	1770	Asia	Myanmar	SAMN15594693	Webb et al., Sci Rep, 2020
H101180656	2010	707	Africa	Nigeria	ERR298772	Chewapreecha et al 2017

G1467	1978	246	Oceania	Papua New Guinea	ERR311036	Chewapreecha et al 2017
MSHR0141	1992	274	Oceania	Papua New Guinea	ERR311041	Chewapreecha et al 2017
MSHR2434	2006	515	Oceania	Papua New Guinea	ERR539760	Chewapreecha et al 2017
MK_441	1990	57	Asia	Philippines	ERR298773	Chewapreecha et al 2017
MK_451	1990	98	Asia	Philippines	ERR298774	Chewapreecha et al 2017
H110440887	2011	unknown	Asia	Philippines	ERR298777	Chewapreecha et al 2017
H0929	1998	92	Americas	Puerto_Rico	ERR311039	Chewapreecha et al 2017
F3253	1982	95	Americas	Puerto_Rico	ERR311035	Chewapreecha et al 2017
MSHR6975	1935	51	Asia	Singapore	ERR298353	Chewapreecha et al 2017
2769a	2001	33	Asia	Thailand	ERR162621	Chewapreecha et al 2017
2698a	2001	34	Asia	Thailand	ERR162620	Chewapreecha et al 2017
2659a	2001	54	Asia	Thailand	ERR162614	Chewapreecha et al 2017
E361	1998	230	Asia	Thailand	ERR162626	Chewapreecha et al 2017
137	2008	67	Asia	Vietnam	ERR178256	Chewapreecha et al 2017
720	2009	67	Asia	Vietnam	ERR178260	Chewapreecha et al 2017
532	2009	163	Asia	Vietnam	ERR178258	Chewapreecha et al 2017
MSHR6973	1953	288	Asia	Vietnam	ERR298351	Chewapreecha et al 2017
1097	2010	367	Asia	Vietnam	ERR178268	Chewapreecha et al 2017
683	2009	871	Asia	Vietnam	ERR178259	Chewapreecha et al 2017
1040	2010	884	Asia	Vietnam	ERR178261	Chewapreecha et al 2017
1042	2010	unknown	Asia	Vietnam	ERR178262	Chewapreecha et al 2017
H065460522	2007	unknown	Americas	Virgin_Islands	ERR298778	Chewapreecha et al 2017
MSHR5087		881	Asia	Borneo	SAMN32784011	Podin et al 2014; Hall et al 2023
MSHR5089		881	Asia	Borneo	SRR2975737	Podin et al 2014; Hall et al 2023
MSHR5093		881	Asia	Borneo	SRR2975738	Podin et al 2014; Hall et al 2023
MSHR5095		881	Asia	Borneo	SRR11649196	Podin et al 2014; Hall et al 2023

MSHR5105		881	Asia	Borneo	SRR2975741	Podin et al 2014; Hall et al 2023
Y2019	2019	881	Asia	Bangladesh	PRJNA1077088	This study
CS6260	2015	56	Asia	Bangladesh	PRJNA1077088	This study
CS2317	2009	756	Asia	Bangladesh	PRJNA1077088	This study
CS60	2015	486	Asia	Bangladesh	PRJNA1077088	This study
CS4987	2015	188	Asia	Bangladesh	PRJNA1077088	This study
CS5414	2015	756	Asia	Bangladesh	PRJNA1077088	This study
CS89	2015	1007	Asia	Bangladesh	PRJNA1077088	This study
CS90	2014	1007	Asia	Bangladesh	PRJNA1077088	This study
NB	2010	1005	Asia	Bangladesh	PRJNA1077088	This study
K35	2012	1005	Asia	Bangladesh	PRJNA1077088	This study
K23	2012	1005	Asia	Bangladesh	PRJNA1077088	This study

Supplementary Figure 1

Map of **A)** South and Southeast Asia region and **B)** Bangladesh with sampling locations shown. The maps were created in datawrapper.de using OpenStreetMap (<https://www.openstreetmap.org/>).

A)

B)

