

Genotyping of *Mycoplasma genitalium* suggests de novo acquisition of antimicrobial resistance, Queensland, Australia

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1 **Title:** Genotyping of *Mycoplasma genitalium* suggests *de novo* acquisition of antimicrobial
2 resistance, Queensland, Australia

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9 **Running title:** *Mycoplasma genitalium* genotyping

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11 **Keywords:** *Mycoplasma genitalium*, Australia, genotyping, *mgpB*, MG309, antimicrobial
12 resistance (AMR)

13 **Text.**

14 *Mycoplasma genitalium* is a sexually-transmitted organism that causes non-gonococcal
15 urethritis in men and pelvic inflammatory disease in women. In Australia, there is concern
16 over the emergence and spread of antimicrobial resistance (AMR) in *M. genitalium* and while
17 high levels of AMR have been reported in men who have sex with men (1, 2), it is not clear if
18 AMR is specific to a particular strain, nor is there any information on the diversity of *M.*
19 *genitalium* strains in Australia.

20 We successfully genotyped 89/111 *M. genitalium*-positive samples collected from 2013-2019
21 (majority of samples from 2017-2019) obtained from different patients as part of a previous
22 study (1). We also genotyped eight additional samples from four patients to investigate

23 changes in genotypes over time. Genotyping consisted of PCR and Sanger sequencing of the
24 *mgpB* and MG_309, as described elsewhere (3-5). Sequence typing was compared to
25 epidemiological data and the presence of macrolide and *parC* quinolone AMR markers as
26 previously described (1).

27

28 Of the 89 individual patient samples, we identified 39 *mgpB* sequence types, of which 21
29 were previously identified and 18 were novel i.e. not previously published or available within
30 GenBank. Of the novel *mgpB* types, 15 were identified only once in our patients, two
31 occurred twice and one was identified across five different samples. Similarly, for MG_309,
32 we identified 23 sequence types, of which eight were novel repeat number variants and one
33 was a new strain type. Novel *mgpB* and MG_309 sequences were deposited into GenBank
34 (accession numbers: MT476043 - MT476061). For the purposes of this study, the *mgpB* and
35 MG_309 data were combined to assign a genotype for each sample, as previously described
36 (6).

37 Results of *M. genitalium* genotyping for individual patient samples can be seen in Figure 1.
38 In brief, we saw 68 different genotypes across 60 males and 29 females, and they were
39 observed in 1 to 5 samples each (average 1.3 samples per genotype). While the presence or
40 absence of resistance markers to macrolides and/or quinolones were stable for some
41 genotypes, in most cases the specific AMR mutations showed a more random distribution,
42 both within and across different genotypes; For example, the two samples belonging to
43 genotype 61 had identical macrolide and quinolone AMR profiles, whereas genotypes 8 (n =
44 5) and 30 (n = 5) both comprised samples with wildtype and mutant *parC* sequences (Figure
45 1). Moreover, the most common *parC* resistance mutation, S83I, was observed across 32
46 different genotypes, followed by D87N (7 genotypes), D87Y (3 genotypes). When comparing

47 gender, specimen site and location, we did not see any correlation with *M. genitalium*
48 genotype. When looking at consecutive samples from the same patient, we observed no
49 change in genotype and AMR profiles in three of the four patients, consistent with chronic *M.*
50 *genitalium* infection or re-infection with the same strain (days between samples ranged from
51 8 to 99 days). In one patient, we observed different genotypes in samples collected 104 days
52 apart, suggesting re-infection with a different *M. genitalium* strain.

53 It should be noted that the genotyping loci used here includes an adhesin (*mgpB*) and an
54 immunogenic, surface-exposed lipoprotein (MG_309) (7, 8) which are likely to be under
55 greater selective pressure to change as they are in constant contact with the host immune
56 system during infection. The highly diverse genotypes/strains reported here are consistent
57 with previous reports from geographically diverse regions of the world (4, 9, 10). Within our
58 study, the lack of association between genotypes and AMR suggest that there are potential
59 inherent limitations in utilising this genotyping scheme for ongoing use in our local
60 population. Additional whole genome sequencing studies may be warranted to fully
61 understand the emergence and spread of *M. genitalium* in Queensland, Australia, both at the
62 population level and within specific patient groups.

63 In summary, we confirm the presence of a wide range of *M. genitalium* genotypes in
64 Queensland, Australia. Geographically and temporally diverse samples contained the same
65 genotype, suggesting that there is ongoing transmission of some strains; however, the
66 variation in AMR mutations across genotypes is highly suggestive of *de novo* acquisition of
67 resistance, rather than being underpinned by clonal spread of specific resistant strains.

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69 provision of samples for this study. This study was approved by the Children's Health
70 Queensland human research ethics committee (HREC/12/QRCH/139).

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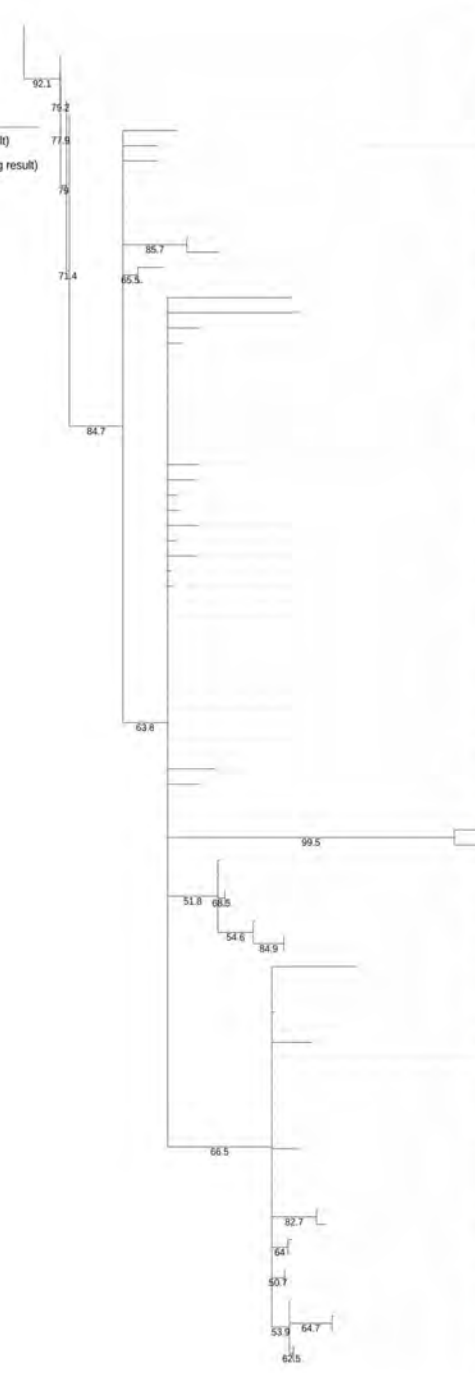
104 **Figure 1.** Neighbor joining tree of 89 *Mycoplasma genitalium* genotyping from individual patient
105 samples. Genotypes were determined based on the presence of SNPs in the *mgpB* gene fragment and
106 tandem repeat unit length of the MG_309 gene. Concatenated sequences were used to construct the
107 phylogenetic tree, including results of the ResistancePlus MG macrolide resistance screening and
108 Sanger sequencing of *parC* for detection of quinolone resistance.

109

Tree scale: 0.001

Location/Region
 South East Queensland
 Northern Queensland

AMR
 Macrolide Resistant (ResistancePlus MG result)
 Quinolone Resistant (parC Sanger sequencing result)



Sample no.	Collection yr	Gender	Specimen site	Location	AMR—Macrolide	AMR—Quinolone	Genotype
29	2017	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87N 41
30	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 41
2	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 42
15	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 42
31	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 60
12	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 21
82	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 1
54	2017	F	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 57
83	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 65
16	2016	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 7
58	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 23
63	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 28
45	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 13
47	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 39
7	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 66
66	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 49
81	2017	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 68
69	2017	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 27
88	2018	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 18
56	2017	F	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 30
52	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 30
25	2016	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 30
1	2013	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 30
6	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 30
40	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 30
42	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 30
50	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87N 30
36	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 29
72	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 29
71	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 15
78	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 32
17	2016	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87N 37
87	2018	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 48
8	2017	F	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 64
24	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 36
4	2016	F	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87H 55
62	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 59
34	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 67
76	2017	M	UNKNOWN	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 43
3	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 8
43	2017	F	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 8
23	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 8
48	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 8
68	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 8
73	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87Y 8
84	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 58
37	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 58
74	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87N 53
80	2017	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 56
53	2017	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87Y 5
10	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 25
55	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 33
67	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 47
39	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 12
51	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87N 61
86	2018	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 20
5	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 17
35	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 17
64	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	D87N 35
85	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 10
11	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 7
77	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 9
57	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 44
28	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 29
9	2016	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 46
49	2017	F	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 46
79	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 46
60	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 62
13	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83R 45
33	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 11
38	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 51
41	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 0
46	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 72
44	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 59
19	2016	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 59
75	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 4
20	2017	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 19
26	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 16
61	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 16
65	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 40
59	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 40
70	2017	F	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 4
32	2017	M	RECTAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 4
89	2019	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 19
18	2016	F	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 16
27	2017	F	CERVIX	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 16
14	2016	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 40
21	2017	M	URINE	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 40
22	2017	F	VAGINAL	South East Queensland	Macrolide Resistant	Quinolone Resistant	S83I 40