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Border Region Surveillance of Malaria Drug Resistance, Northern Burundi, 2023–2024

Appendix

Appendix Table 1. Distribution of *Plasmodium falciparum* resistance-associated mutations across eight sentinel sites in northern Burundi*

| Gene | Codon | AA | KIRUNDO | | | NGOZI | | | Total | Total | p-value |
|------------------|-------|-----------|------------|-----------|------------|-----------|-----------|-----------|------------|------------|---------|
| | | | KIRUNDO | VUMBI | Total | BUYE | KIREMBA | NGOZI | | | |
| No. samples | | | 155 | 62 | 217 | 76 | 30 | 42 | 148 | 365 | |
| <i>Pfkelch13</i> | WT | - | 149 (96.1) | 62 (100) | 211 (97.2) | 74 (97.4) | 29 (96.7) | 42 (100) | 145 (98.0) | 356 (97.5) | 0.5 |
| | 513 | R>S | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |
| | 578 | A>S | 2 (1.3) | 0 | 2 (0.9) | 1 (1.3) | 1 (3.3) | 0 | 2 (1.4) | 4 (1.1) | |
| | 626 | A>S | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |
| | 644 | H>R | 0 | 0 | 0 | 1 (1.3) | 0 | 0 | 1 (0.7) | 1 (0.3) | |
| | 672 | N>H | 2 (1.3) | 0 | 2 (0.9) | 0 | 0 | 0 | 0 | 2 (0.5) | |
| <i>Pfprt</i> | 72 | C | 155 (100) | 61 (98.4) | 216 (99.5) | 73 (96.1) | 30 (100) | 42 (100) | 145 (98.0) | 361 (98.9) | 0.16 |
| | | S | 0 | 1 (1.6) | 1 (0.5) | 3 (3.9) | 0 | 0 | 3 (2.0) | 4 (1.1) | |
| | 74 | M | 8 (5.2) | 5 (8.1) | 13 (6.0) | 1 (1.3) | 1 (3.3) | 4 (9.5) | 6 (4.1) | 19 (5.2) | 0.4 |
| | | I | 147 (94.8) | 57 (91.9) | 204 (94.0) | 75 (98.7) | 29 (96.7) | 38 (90.5) | 142 (95.9) | 346 (94.8) | |
| | 75 | N | 8 (5.2) | 5 (8.1) | 13 (6.0) | 1 (1.3) | 1 (3.3) | 4 (9.5) | 6 (4.1) | 19 (5.2) | 0.4 |
| | | E | 147 (94.8) | 57 (91.9) | 204 (94.0) | 75 (98.7) | 29 (96.7) | 38 (90.5) | 142 (95.9) | 346 (94.8) | |
| | 76 | K | 8 (5.2) | 5 (8.1) | 13 (6.0) | 1 (1.3) | 1 (3.3) | 4 (9.5) | 6 (4.1) | 19 (5.2) | 0.4 |
| | | T | 147 (94.8) | 57 (91.9) | 204 (94.0) | 75 (98.7) | 29 (96.7) | 38 (90.5) | 142 (95.9) | 346 (94.8) | |
| | 93 | T | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - |
| | | S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 97 | H | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - |
| | | Y | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 145 | F | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - |
| | | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 218 | I | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - |
| | | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 343 | M | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - |
| | | L | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 350 | C | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - |
| | | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 353 | G | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - | |

| Gene | Codon | AA | KIRUNDO | | | NGOZI | | | | Total | p-value | |
|---------------|-------------|------------|------------|------------|------------|------------|-----------|------------|-------------------|-------------------|-------------------|-----|
| | | | KIRUNDO | VUMBI | Total | BUYE | KIREMBA | NGOZI | Total | | | |
| | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| <i>Pfmdr1</i> | 356 | I | 142 (91.6) | 57 (91.9) | 199 (91.7) | 71 (93.4) | 28 (93.3) | 32 (76.2) | 131 (88.5) | 330 (90.4) | 0.31 | |
| | | T | 13 (8.4) | 5 (8.1) | 18 (8.3) | 5 (6.6) | 2 (6.7) | 10 (23.8) | 17 (11.5) | 35 (9.6) | | |
| | 86 | N | 137 (88.4) | 52 (83.9) | 189 (87.1) | 73 (96.1) | 29 (96.7) | 38 (90.5) | 140 (94.6) | 329 (90.1) | 0.02 | |
| | | Y | 18 (11.6) | 10 (16.1) | 28 (12.9) | 3 (3.9) | 1 (3.3) | 4 (9.5) | 8 (5.4) | 36 (9.9) | | |
| | 184 | Y | 58 (37.4) | 30 (48.4) | 88 (40.6) | 23 (30.3) | 12 (40.0) | 21 (50.0) | 56 (37.8) | 144 (39.5) | 0.6 | |
| | | F | 97 (62.6) | 32 (51.6) | 129 (59.4) | 53 (69.7) | 18 (60.0) | 21 (50.0) | 92 (62.2) | 221 (60.5) | | |
| 1034 | S | 153 (98.7) | 60 (96.8) | 213 (98.2) | 74 (97.4) | 30 (100) | 41 (97.6) | 145 (98.0) | 358 (98.1) | 0.9 | | |
| | C | 2 (1.3) | 2 (3.2) | 4 (1.8) | 2 (2.6) | 0 | 1 (2.4) | 3 (2.0) | 7 (1.9) | | | |
| 1042 | N | 153 (98.7) | 61 (98.4) | 214 (98.6) | 74 (97.4) | 30 (100) | 41 (97.6) | 145 (98.0) | 359 (98.4) | 0.6 | | |
| | D | 2 (1.3) | 1 (1.6) | 3 (1.4) | 2 (2.6) | 0 | 1 (2.4) | 3 (2.0) | 6 (1.6) | | | |
| 1246 | D | 155 (100) | 62 (100) | 217 (100) | 74 (97.4) | 30 (100) | 41 (97.6) | 145 (98.0) | 362 (99.2) | 0.03 | | |
| | Y | 0 | 0 | 0 | 2 (2.6) | 0 | 1 (2.4) | 3 (2.0) | 3 (0.8) | | | |
| <i>dhfr</i> | 16 | A | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - | |
| | | V | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | 50 | C | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - | |
| | | R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | 51 | N | 10 (6.5) | 2 (3.2) | 13 (6.0) | 1 (1.3) | 0 | 0 | 1 (0.7) | 14 (3.8) | 0.01 | |
| | | I | 145 (93.5) | 59 (95.2) | 204 (94.0) | 75 (98.7) | 30 (100) | 42 (100) | 147 (99.3) | 351 (96.2) | | |
| | 59 | C | 10 (6.5) | 4 (6.5) | 14 (6.5) | 1 (1.3) | 1 (3.3) | 2 (4.8) | 4 (2.7) | 18 (4.9) | 0.1 | |
| | | R | 145 (93.5) | 58 (93.5) | 203 (93.5) | 75 (98.7) | 29 (96.7) | 40 (95.2) | 144 (97.3) | 347 (95.1) | | |
| | 108 | S | 8 (5.2) | 3 (4.8) | 11 (5.1) | 1 (1.3) | 0 | 0 | 1 (0.7) | 12 (3.3) | 0.02 | |
| | | N | 147 (94.8) | 59 (95.2) | 206 (94.9) | 75 (98.7) | 30 (100) | 42 (100) | 147 (99.3) | 353 (96.7) | | |
| | 164 | I | 150 (97.0) | 61 (98.4) | 211 (97.2) | 72 (94.7) | 30 (100) | 41 (97.6) | 143 (96.6) | 354 (97.0) | 0.7 | |
| | | L | 5 (3.2) | 1 (1.6) | 6 (2.8) | 4 (5.3) | 0 | 1 (2.4) | 5 (3.4) | 11 (3.0) | | |
| | <i>dhps</i> | 431 | I | 155 (100) | 62 (100) | 217 (100) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 365 (100) | - |
| | | | V | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | 436 | S | 153 (98.7) | 53 (85.5) | 206 (94.9) | 76 (100) | 29 (96.7) | 40 (95.2) | 145 (98.0) | 351 (96.2) | 0.1 |
| | | | A | 2 (1.3) | 9 (14.5) | 11 (5.1) | 0 | 1 (3.3) | 2 (4.8) | 3 (2.0) | 14 (3.8) | |
| | | 437 | A | 9 (5.8) | 10 (16.1) | 19 (8.8) | 4 (5.3) | 1 (3.3) | 6 (14.3) | 11 (7.4) | 30 (8.2) | 0.6 |
| | | | G | 146 (94.2) | 52 (83.9) | 198 (91.2) | 72 (94.7) | 29 (96.7) | 36 (85.7) | 137 (92.6) | 335 (91.8) | |
| 540 | | K | 5 (3.2) | 7 (11.3) | 14 (6.5) | 4 (5.3) | 1 (3.3) | 4 (9.5) | 9 (6.1) | 21 (5.8) | 0.8 | |
| | | E | 150 (96.8) | 55 (88.7) | 203 (93.5) | 72 (94.7) | 29 (96.7) | 38 (90.5) | 139 (93.9) | 344 (94.2) | | |
| 581 | | A | 76 (49.0) | 31 (50.0) | 107 (49.3) | 37 (48.7) | 14 (46.7) | 23 (54.8) | 74 (50.0) | 181 (49.6) | 0.9 | |
| | | G | 79 (51.0) | 51 (50.0) | 110 (50.7) | 39 (51.3) | 16 (53.3) | 19 (45.2) | 74 (50.0) | 184 (50.4) | | |
| 613 | | A | 154 (99.4) | 62 (100) | 216 (99.5) | 76 (100) | 30 (100) | 42 (100) | 148 (100) | 364 (99.7) | 0.4 | |
| | | S | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | | |

*The table presents the frequency of amino acid variants in *pfkelch13*, *pfprt*, *pfmdr1*, *dhfr*, and *dhps* genes across individual sites grouped by province (Kirundo and Ngozi), and for the entire dataset (N = 365). Mutant and wild-type alleles are listed for each codon. Site-level and overall p-values (Chi-square or Fisher's exact test, as appropriate) compare allele frequencies between health centers and provinces. P-values show difference in proportions between provinces. Significant p-values (p < 0.05) are shown in bold. Total percentages are calculated relative to the number of samples successfully genotyped per locus.

Appendix Table 2. Distribution of single nucleotide polymorphisms (SNPs) and haplotypes in *pfkelch13*, *pfprt*, *pfmdr1*, *dhfr*, and *dhps* genes across eight sentinel sites in northern Burundi*

| Gene | Allele | | KIRUNDO | | | NGOZI | | | Total | Total | p-value |
|-------------------|------------------------|---------------|------------|-----------|------------|-----------|-----------|-----------|------------|------------|-------------|
| | | | KIRUNDO | VUMBI | Total | BUYE | KIREMBA | NGOZI | | | |
| No. of sample (%) | | | 155 | 62 | 217 | 76 | 30 | 42 | 148 | 365 | - |
| <i>Pfkelch13</i> | WT | - | 149 (96.1) | 62 (100) | 211 (97.2) | 74 (97.4) | 29 (96.7) | 42 (100) | 145 (98.0) | 356 (97.5) | 0.5 |
| | 513 | R>S | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |
| | 578 | A>S | 2 (1.3) | 0 | 2 (0.9) | 1 (1.3) | 1 (3.3) | 0 | 2 (1.4) | 4 (1.1) | |
| | 626 | A>S | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |
| | 644 | H>R | 0 | 0 | 0 | 1 (1.3) | 0 | 0 | 1 (0.7) | 1 (0.3) | |
| | 672 | N>H | 2 (1.3) | 0 | 2 (0.9) | 0 | 0 | 0 | 0 | 2 (0.5) | |
| <i>Pfprt</i> | WT | CVMNKTHFIMCGI | 8 (5.2) | 5 (8.1) | 13 (6.0) | 1 (1.3) | 1 (3.3) | 3 (7.1) | 5 (3.4) | 18 (4.9) | 0.25 |
| | 72S/76T | SVMNTHFIMCGI | 0 | 1 (1.6) | 1 (0.5) | 3 (3.9) | 0 | 0 | 3 (2.0) | 4 (1.1) | |
| | 74I/75E/76T | CVIETTHFIMCGI | 134 (69.0) | 51 (82.3) | 185 (85.3) | 67 (88.2) | 27 (90.0) | 29 (82.3) | 123 (83.1) | 308 (84.4) | |
| | 356T | CVMNKTHFIMCGT | 0 | 0 | 0 | 0 | 0 | 1 (2.4) | 1 (0.7) | 1 (0.3) | |
| | 74I/75E/76T/356T | CVIETTHFIMCGT | 13 (8.4) | 5 (8.1) | 18 (8.3) | 5 (6.6) | 2 (6.7) | 9 (21.4) | 16 (10.8) | 34 (9.3) | |
| <i>Pfmdr1</i> | WT | NYSND | 43 (27.7) | 23 (37.1) | 66 (30.4) | 20 (26.3) | 11 (36.7) | 17 (40.5) | 48 (32.4) | 114 (31.2) | 0.03 |
| | 86Y | YYSND | 15 (9.7) | 6 (9.7) | 21 (9.7) | 3 (3.9) | 1 (3.3) | 4 (9.5) | 8 (5.4) | 29 (7.9) | |
| | 184F | NFSND | 92 (59.4) | 28 (45.2) | 120 (55.3) | 51 (67.1) | 18 (60.0) | 20 (47.6) | 89 (60.1) | 209 (57.3) | |
| | 86Y/184F | YFSND | 3 (1.9) | 3 (4.8) | 6 (2.8) | 0 | 0 | 0 | 0 | 6 (1.6) | |
| | 86Y/1034C | YYCND | 0 | 1 (1.6) | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |
| | 184F/1034C/1042D | NFCDD | 2 (1.3) | 1 (1.6) | 3 (1.4) | 0 | 0 | 0 | 0 | 3 (0.8) | |
| | 184F/1034C/1042D/1246Y | NFCDY | 0 | 0 | 0 | 2 (2.6) | 0 | 1 (2.4) | 3 (2.0) | 3 (0.8) | |
| <i>dhfr</i> | WT | ACNCSI | 8 (5.2) | 3 (4.8) | 11 (5.1) | 1 (1.3) | 0 | 0 | 1 (0.7) | 12 (3.3) | 0.2 |
| | 108N | ACNCNI | 2 (1.3) | 0 | 2 (0.9) | 0 | 0 | 0 | 0 | 2 (0.5) | |
| | 51I/108N | ACICNI | 0 | 1 (1.6) | 1 (0.5) | 0 | 1 (3.3) | 2 (4.8) | 3 (2.0) | 4 (1.1) | |
| | 51I/59R/108N | ACIRNI | 140 (90.3) | 57 (91.9) | 197 (90.8) | 71 (93.4) | 29 (96.7) | 39 (92.9) | 139 (93.9) | 336 (92.1) | |
| | 51I/59R/108N/164L | ACIRNL | 5 (5.2) | 1 (1.6) | 11 (5.1) | 4 (5.3) | 0 | 1 (2.4) | 1 (0.7) | 12 (3.3) | |
| <i>dhps</i> | WT | ISAKAA | 5 (3.2) | 7 (11.3) | 12 (5.5) | 4 (5.3) | 1 (3.3) | 4 (9.5) | 9 (6.1) | 21 (5.8) | 0.6 |
| | 540E | ISAEAA | 4 (2.6) | 3 (4.8) | 7 (3.2) | 0 | 0 | 2 (4.8) | 2 (1.4) | 9 (2.5) | |
| | 437G/540E | ISGEAA | 67 (43.2) | 19 (30.6) | 86 (39.6) | 33 (43.4) | 13 (43.3) | 16 (38.1) | 62 (41.9) | 148 (40.5) | |
| | 436A/437G/540E | IAGEAA | 0 | 2 (3.2) | 2 (0.8) | 0 | 0 | 1 (2.4) | 1 (0.7) | 3 (0.8) | |
| | 437G/540E/581G | ISGEGA | 76 (49.0) | 24 (38.7) | 100 (46.1) | 39 (51.3) | 15 (50.0) | 18 (42.9) | 72 (48.6) | 172 (47.1) | |
| | 436A/437G/540E/581G | IAGEGA | 2 (1.3) | 7 (11.3) | 9 (4.1) | 0 | 1 (3.3) | 1 (2.4) | 2 (1.4) | 11 (3.0) | |
| | 437G/540E/581G/613S | ISGEGS | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |

| Gene | Allele | | KIRUNDO | | | NGOZI | | | Total | Total | p-value |
|------------------|------------------|----------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|---------|
| | | | KIRUNDO | VUMBI | Total | BUYE | KIREMBA | NGOZI | | | |
| <i>dhfr_dhps</i> | Wild-type | WT_WT | 0 | 0 | 0 | 1 (1.3) | 0 | 0 | 1 (0.7) | 1 (0.3%) | 0.2 |
| | Double mutant | 511R/108N_WT | 0 | 1 (1.6) | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |
| | | WT_437G/540E | 5 (3.2) | 1 (1.6) | 6 (2.8) | 0 | 0 | 0 | 0 | 6 (1.6) | |
| | Triple mutant | WT_437G/540E/581G | 3 (1.9) | 1 (1.6) | 4 (1.8) | 0 | 0 | 0 | 0 | 4 (1.1) | |
| | | 511/108N_540E | 0 | 0 | 0 | 0 | 0 | 1 (2.4) | 1 (0.7) | 1 (0.3%) | |
| | | 511/59R/108N_WT | 5 (3.2) | 6 (9.7) | 11 (5.1) | 3 (3.9) | 1 (3.3) | 4 (9.5) | 8 (5.4) | 19 (5.2) | |
| | | 108N_437G/540E | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3%) | |
| | Quadruple mutant | 108N_437G/540E/581G | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3%) | |
| | | 511/59R/108N_540E | 4 (2.6) | 3 (4.8) | 7 (3.2) | 0 | 0 | 1 (2.4) | 1 (0.7) | 8 (2.2) | |
| | | WT_436A/437G/540E/581G | 0 | 1 (1.6) | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3%) | |
| | Quintuple mutant | 511/108N_437G/540E/581G | 0 | 0 | 0 | 0 | 1 (3.3) | 1 (2.4) | 2 (1.4) | 2 (0.5) | |
| | | 511/59R/108N_437G/540E | 58 (37.4) | 17 (27.4) | 75 (34.6) | 30 (39.5) | 13 (43.3) | 16 (38.1) | 59 (39.9) | 134 (36.7) | |
| | Sextuple mutant | 511/59R/108N_436A/437G/540E | 0 | 2 (3.2) | 2 (0.9) | 0 | 0 | 1 (2.4) | 1 (0.7) | 3 (0.8) | |
| | | 511/59R/108N_437G/540E/581G | 70 (45.2) | 23 (37.1) | 93 (42.9) | 38 (50.0) | 14 (46.7) | 16 (38.1) | 68 (45.9) | 161 (44.1) | |
| | | 511/59R/108N/164L_437G/540E | 3 (1.9) | 1 (1.6) | 4 (1.8) | 3 (3.9) | 0 | 0 | 3 (2.0) | 7 (1.9) | |
| | Septuple mutant | 511/59R/108N_436A/437G/540E/581G | 2 (1.3) | 6 (9.7) | 8 (3.7) | 0 | 1 (3.3) | 1 (2.4) | 2 (1.4) | 10 (2.7) | |
| | | 511/59R/108N_437G/540E/581G/613S | 1 (0.6) | 0 | 1 (0.5) | 0 | 0 | 0 | 0 | 1 (0.3) | |
| | | 511/59R/108N/164L_437G/540E/581G | 2 (1.3) | 0 | 2 (0.9) | 1 (1.3) | 0 | 1 (2.4) | 2 (0.9) | 4 (1.1) | |

*The table shows the frequency of point mutations and derived haplotypes conferring resistance to artemisinin and partner drugs in *Plasmodium falciparum*, grouped by province (Kirundo and Ngozi). Wild-type and mutant alleles are indicated for each codon or haplotype. Combined *dhfr/dhps* haplotypes are classified as wild-type, single, double, triple, quadruple, quintuple, or sextuple mutants. Percentages were calculated among successfully genotyped samples for each gene. p-values were derived from Chi-square or Fisher's exact test as appropriate. P-values show difference in proportions between provinces. Statistically significant p-values ($p < 0.05$) are shown in bold.