Emerging artemisinin resistance to *Plasmodium falciparum* endangers malaria control worldwide. Currently, the resistance epicenter is the greater Mekong subregion in Southeast Asia (1). In sub-Saharan Africa, where illnesses and deaths from *P. falciparum* malaria are highest, such resistance may result in disastrous consequences (2). Early detection and close monitoring are therefore crucial.

Artemisinin resistance in *P. falciparum* is associated with nonsynonymous mutations in the Kelch 13 (K13) propeller domain. We found that 12.1% (8/66) of clinical *P. falciparum* isolates from Huye district, Rwanda, exhibited K13 mutations, including R561H, a validated resistance marker. K13 mutations appear to be increasing in this region.

Rwanda achieved substantial reductions in malaria during 2006–2011, partly due to home-based management using artemether/lumefantrine (6). In 2010, at our study site in Huye district, southern Rwanda, we observed a pattern in the *P. falciparum* multidrug resistance: 1 gene suggestive of intense artemether/lumefantrine drug pressure, whereas K13 mutations were absent. However, among *P. falciparum* isolates, 2.5% in 2014 and 4.5% in 2015 harbored K13 variants, including 2 candidate mutations (7,8). A recent report showed the presence of a validated pfkelch13 mutation, R561H, at 2 sites in Rwanda (9). We conducted a cross-sectional molecular surveillance study to update records of the prevalence of K13 variants in Huye among isolates collected in 2019.

The Study

During September–December 2019, we recruited study patients with uncomplicated malaria seeking treatment at the Sovu Health Centre and Kabutare District Hospital, Huye district, Rwanda. Huye district (population ≈390,000) is located on the central plateau of Rwanda (average altitude 1,700 m, yearly rainfall 1,200 mm, mean temperature 19°C). Malaria transmission peaks in October–November and March–May. In 2010, a total of 11.7% of children had microscopically confirmed *Plasmodium* infection (8).

We obtained written informed consent from all participants or from the caregivers for children; we also obtained written assent from participants 7–18 years of age. The study was approved by the Rwanda National Ethics Committee. Eligibility criteria for participants included age >1 year; a positive result on a rapid diagnostic test, SD Bioline Malaria Ag Pf/Pan (Abbott Global Point of Care, https://www.globalpointofcare.abbott); and a fever (axillary temperature ≥37.5°C) at the time they sought treatment or within 48 hours beforehand (self-reported). We collected whole blood in S-Monovette EDTA (ethylenediaminetetraacetic acid–anticoagulant tubes). We used a sensitive, molecular diagnostic method (10) for detection of K13 polymorphisms in *P. falciparum* isolates.

Author affiliations: Charité–Universitätsmedizin Berlin, Berlin, Germany (C. Bergmann, W. van Loon, C. Tacoli, J.C. Jäger, D. Savelberg, F.P. Mockenhaupt); University Teaching Hospital of Butare, Butare, Rwanda (F. Habarugira, E. Rwamugema, D. Mbarushimana, J. Ndoli, A. Sendegeya); Kabutare District Hospital, Butare (F. Nshimiyimana); University of Rwanda, Kigali, Rwanda (C. Bayingana)

DOI: https://doi.org/10.3201/eid2701.203527
acid; Sarstedt, https://www.sarstedt.com) tubes and confirmed malaria diagnosis by microscopy of Giemsa-stained thick blood smears; patients were also seen by a physician. We provided a 3-day regimen of arteether/lumefantrine for treatment, the first dose given under observation. All patients were asked to return after 3 days to evaluate residual parasitemia on Giemsa-stained thick blood smears.

Definite parasite density was counted per 200 leukocytes on Giemsa-stained thick blood smears by 2 independent microscopists, assuming a mean leukocyte count of 8,000/µL. We extracted DNA using a QIAamp DNA Blood Mini kit (Qiagen, https://www.qiagen.com). Plasmodium species were typed by real-time PCRs with commercially available primers and probes for P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi (TIB MolBiol, https://www.tib-molbiol.com) on a Roche LightCycler 480 device (https://lifescience.roche.com). K13 was amplified (codons ≥441≤688) by using nested PCR (3) and sequenced by a commercial provider (Eurofins Genomics, https://www.eurofins-genomics.com). Sequences were aligned to reference K13 3D7–1343700 (PlasmoDB, https://plasmodb.org) by using Geneious Prime version 2020.1 (https://www.geneious.com). We used R version 3.6.3 (https://cran.r-project.org) for statistical analysis and a binomial logistic regression model to estimate the time-trend of nonsynonymous mutations (p<0.05).

Of 90 patients included in the study, 74 tested positive by microscopy and PCR and 2 by PCR only. Of 90 patients included in the study, 74 tested positive by microscopy and PCR and 2 by PCR only. Among these patients, 51.3% (39/76) were female and 2–69 years). Among these patients, 51.3% (39/76) were female and 2–69 years. Of note, the validated marker R561H alone occurred in 1.8; p = 0.003) over the previous decade compared with their absence in 2010 and 4.5% prevalence in 2015 (7). Of note, the validated marker R561H alone occurred in 4.5% of the isolates collected in 2019. Recent studies report 1%–3.5% of nonsynonymous K13 polymorphisms in parasite isolates from East Africa (10), whereas during 2013–2015 in Rwanda, this figure was 6.9% (9).
The R561H artemisinin resistance mutation is regularly observed across Asia (10). A recent study that reported R561H in 7.4% of isolates collected during 2013–2015 in central Rwanda and 0.7% of isolates in southern Rwanda suggested that this mutation emerged independently and specifically from Asia 561H strains. We do not have data in our study to support this. None of the K13 variant parasites showed delayed clearance in our study, which may be due to the partner drug lumefantrine still being effective, similar to observations in Southeast Asia (11). In addition, the absence of delayed parasite clearance despite K13 mutations may reflect partial immunity contributing to parasite elimination (12).

We found other nonsynonymous polymorphisms only once among the isolates tested. C469F and A675V are considered artemisinin resistance candidate

| Year | No. sequenced isolates | No. (%) isolates with nonsynonymous mutations | Amino acid changes and nucleotide changes |
|------|------------------------|---------------------------------------------|-----------------------------------------|
| 2010 | 75                     | 0                                           | Not applicable                          |
| 2014 | 81                     | 2 (2.5)                                     | V555A, A626S                            |
| 2015 | 66                     | 3 (4.5)                                     | P574L†, D648H, A675V†                   |
| 2019 | 66                     | 8 (12.1)                                    | C469F†, G533A, V555A, R561H† (3†), A578S, A675V† |

*Data during 2010–2015 derived from Tacoli et al. (7).
†Candidate mutations for artemisinin resistance.
‡Validated mutation for artemisinin resistance (4).
mutations (4) and have previously been seen in East Africa (7,13,14). G533A and V555A have also been previously reported in Africa but have not yet been evaluated for resistance (7,13). A578S is a common K13 polymorphism across Africa but is not linked to artemisinin resistance (1). Our study has clear limitations. Data from only 2 healthcare facilities, with limited catchment areas, were included. Adherence to treatment was assessed by patient self-report, and drug susceptibility testing was not performed. Future research should include ring-stage susceptibility assays to contribute to understanding the role of K13 mutations in Africa. Separate testing for each drug in a combination for efficacy and continued surveillance for antimicrobial resistance are needed.

Our results show that K13 mutations are present in Rwanda and that their prevalence in *Plasmodium falciparum* malaria patients in the Huye District increased from 0% in 2010 to >12% in 2019. The validated artemisinin resistance mutation R561H occurs in 4.5% of *P. falciparum* isolates being transmitted in this area. The emergence of artemisinin resistance–related mutations in Rwanda is alarming because it might indicate developing resistance against commonly used antimalarials in this region. Countermeasures need to be considered early, potentially including 3-drug antimalarial combinations (2).

Acknowledgments
We are grateful to the staff of Sovu Health Centre and Kabutare District Hospital for their collaboration and help for over 10 years.

This study was financially supported by grant GRK2046 from the German Research Foundation (DFG), which also supported W.L.C. Bergmann was supported by DFG grant GRK2290. The funding bodies had no role in designing the study, collecting, analyzing, or interpreting data, or writing the manuscript.

About the Author
Ms. Bergmann is a medical student at Charité–Universitätsmedizin Berlin, interested in infectious disease epidemiology and tropical diseases. This manuscript forms part of her medical doctoral thesis.

References
1. Ménard D, Khim N, Beghain J, Adegnik AA, Shafui-Alam M, Amedou O, et al. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. N Engl J Med. 2016;374:2453–64. https://doi.org/10.1056/NEJMoai1513137
2. Hanboonkunupakarn B, White NJ. The threat of antimalarial drug resistance. Trop Dis Travel Med Vaccines. 2015;2:10. https://doi.org/10.1186/s40794-016-0027-8
3. Aricay F, Witkowski B, Amarutunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;505:50–5. https://doi.org/10.1038/nature12876
4. World Health Organisation Global Malaria Programme. Status report on artemisinin resistance and artemisinin-based combination therapy efficacy. Geneva: The Organisation; 2018 Aug [cited 10 May 2020]. https://apps.who.int/iris/bitstream/handle/10665/274362/WHO-CDS-GMP-2018.18-eng.pdf
5. Amato R, Miotto O, Woodrow CJ, Almagro-Garcia J, Sinha I, Campino S, et al. Genomic epidemiology of artemisinin resistant malaria. Elife. 2016;5:e08714. https://doi.org/10.7554/eLife.08714
6. Uwimana A, Penkunus MJ, Nisingizwe MP, Uyizeye D, Hakizimana D, Musanabaganwa C, et al. Expanding home-based management of malaria to all age groups in Rwanda: analysis of accessibility and facility-level time-series data. Trans R Soc Trop Med Hyg. 2018;112:513–21. https://doi.org/10.1093/trstmh/try093
7. Tacoli C, Gai PP, Bayingana C, Siff K, Geus D, Ndolli J, et al. Artemisinin resistance–associated K13 polymorphisms of *Plasmodium falciparum* in southern Rwanda, 2010–2015. Am J Trop Med Hyg. 2016;95:1090–3. https://doi.org/10.4269/ajtmh.16-0483
8. Gahutu JB, Steinhöfer C, Shyirambere C, Zeile I, Cwinya-Ay N, Danquah I, et al. Prevalence and risk factors of malaria among children in southern highland Rwanda. Malar J. 2011;10:134. https://doi.org/10.1186/1475-2875-10-134
9. Uwimana A, Leegrand E, Stokes BH, Ndikumana JLM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum kelch13 R561H* mutant parasites in Rwanda. Nat Med. 2020;26:1602–8. https://doi.org/10.1038/s41591-020-1005-2
10. Ocan M, Akena D, Nsobya S, Kamya MR, Senono R, Kinengyere AA, et al. K13-propeller gene polymorphisms in *Plasmodium falciparum* parasite population in malaria affected countries: a systematic review of prevalence and risk factors. Malar J. 2019;18:60. https://doi.org/10.1186/s12936-019-2701-6
11. Ashley EA, Dhorda M, Fairhurst RM, Amarutunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. Nat Engl J Med. 2014;371:411–23. https://doi.org/10.1056/NEJMoai1314981
12. Ataide R, Ashley EA, Powell R, Chan JA, Malloy MJ, O’Flaherty K, et al. Host immunity to *Plasmodium falciparum* and the assessment of emerging artemisinin resistance in a multinational cohort. Proc Natl Acad Sci U S A. 2017;114:3515–20. https://doi.org/10.1073/pnas.1615875114
13. Conrad MD, Nsobya SL, Rosenthal PJ. The diversity of the *Plasmodium falciparum* K13 propeller domain did not increase after implementation of artemisinin-based combination therapy in Uganda. Antimicrob Agents Chemother. 2019;63:e01234-19. https://doi.org/10.1128/AAC.01234-19
14. Ikeda M, Kaneko M, Tachibana SI, Balikagala B, Sakurai-Yatsushiro M, Yatsushiro S, et al. Artemisinin-resistant *Plasmodium falciparum* with high survival rates, Uganda, 2014–2016. Emerg Infect Dis. 2018;24:718–26. https://doi.org/10.3201/eid2404.170141

Address for correspondence: Welmoed van Loon, Institute of Tropical Medicine and International Health, Charité-Universitätsmedizin Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany; email: welmoed.vanloon@gmail.com.