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# Geographic Heterogeneity in Antimicrobial Resistance Profiles of *Vibrio cholerae* O1 in the Democratic Republic of the Congo: A Comparative Analysis of North Kivu, South Kivu, and Tanganyika Provinces.

*Hétérogénéité géographique des profils de résistance aux antimicrobiens de Vibrio cholerae O1 en République démocratique du Congo : analyse comparative des provinces du Nord-Kivu, du Sud-Kivu et du Tanganyika.*

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***ABSTRACT :***

***Antimicrobial resistance (AMR) in Vibrio cholerae represents a major threat to cholera control efforts in Africa. However, data on the geographic distribution of AMR patterns in the Democratic Republic of the Congo (DRC) remain limited and fragmented. This study aimed to describe the geographic heterogeneity of AMR profiles among V. cholerae O1 isolates from three cholera hotspot provinces in eastern DRC and to identify associated determinants. We conducted a repeated analytical cross-sectional study including 3,676 V. cholerae O1 isolates obtained from 25,421 stool samples collected between 2007 and 2023 by the National Cholera Reference Laboratory in Goma, across three eastern DRC provinces (North Kivu, South Kivu, and Tanganyika). Antimicrobial resistance patterns were analyzed using two complementary approaches: (i) a generalized linear mixed-effects model (GLMM) with a random intercept for health zones to quantify spatial heterogeneity through the intraclass correlation coefficient (ICC) and to test Province × Serotype interactions using likelihood ratio tests; and (ii) Firth's penalized logistic regression, restricted to the common period 2021–2022, to estimate adjusted odds ratios (aORs) for erythromycin resistance while addressing quasi-complete separation. Analyses were performed using R version 4.3.3. Resistance was nearly universal to ampicillin (99.8%), chloramphenicol (100%), cotrimoxazole (100%), and nalidixic acid (99.9%), while no resistance was detected to tetracycline, doxycycline, norfloxacin, or azithromycin. Only erythromycin resistance showed marked geographic heterogeneity, with a clear north-to-south gradient (28.2% in North Kivu, 65.4% in South Kivu, and 86.5% in Tanganyika; p < 0.001) and extreme within-province variability (0–97.9% across health zones). Health zone-level variation accounted for 56.7% of the total variance (ICC = 0.567). After adjustment for temporal effects, geographic differences remained significant, with higher odds of erythromycin resistance in South Kivu (adjusted OR = 1.93; 95% CI: 1.31–2.85; p = 0.001) and Tanganyika (adjusted OR = 4.02; 95% CI: 1.89–9.27; p < 0.001). AMR among V. cholerae in the DRC is characterized by near-universal resistance to historically used antibiotics, while susceptibility to doxycycline and azithromycin remains preserved. The marked geographic gradient in erythromycin resistance, substantial within-province heterogeneity, and significant Province × Serotype interaction highlights the need for surveillance at the health zone level to guide locally adapted cholera treatment strategies.***

***Keywords: Vibrio cholerae; antimicrobial resistance; cholera; Democratic Republic of the Congo; mixed-effects models; spatial heterogeneity***

***RESUME :***

***La résistance aux antimicrobiens (RAM) chez Vibrio cholerae constitue une menace majeure pour les efforts de lutte contre le choléra en Afrique. Cependant, les données relatives à la distribution géographique des profils de résistance aux antimicrobiens en République démocratique du Congo (RDC) demeurent limitées et fragmentaires. Cette étude visait à décrire l'hétérogénéité géographique des profils de résistance aux antimicrobiens parmi les isolats de V. cholerae O1 provenant de trois provinces de l'est de la RDC considérées comme des foyers majeurs de choléra, et à identifier les facteurs associés. Nous avons mené une étude transversale analytique répétée portant sur 3 676 isolats de V. cholerae O1 obtenus à partir de 25 421 échantillons de selles collectés entre 2007 et 2023 par le Laboratoire national de référence du choléra à Goma, dans trois provinces de l'est de la RDC (Nord-Kivu, Sud-Kivu et Tanganyika). Les profils de résistance aux antimicrobiens ont été analysés selon deux approches complémentaires : (i) un modèle linéaire généralisé mixte (GLMM), intégrant un intercept aléatoire pour les zones de santé, afin de quantifier l'hétérogénéité spatiale au moyen du coefficient de corrélation intraclasse (CCI) et de tester les interactions Province × Sérotype à l'aide de tests du rapport de vraisemblance ; et (ii) une régression logistique pénalisée de Firth, limitée à la période commune 2021–2022, afin d'estimer les rapports de cotes ajustés (aOR) de la résistance à l'érythromycine, tout en tenant compte du problème de séparation quasi complète. Les analyses ont été réalisées à l'aide du logiciel R, version 4.3.3. La résistance était quasi universelle à l'ampicilline (99,8 %), au chloramphénicol (100 %), au cotrimoxazole (100 %) et à l'acide nalidixique (99,9 %), tandis qu'aucune résistance n'a été détectée à la tétracycline, à la doxycycline, à la norfloxacine ou à l'azithromycine. Seule la résistance à l'érythromycine présentait une hétérogénéité géographique marquée, caractérisée par un gradient net du nord vers le sud (28,2 % au Nord-Kivu, 65,4 % au Sud-Kivu et 86,5 % au Tanganyika ; p < 0,001), ainsi qu'une variabilité intraprovinciale extrême (de 0 à 97,9 % selon les zones de santé). Les variations observées au niveau des zones de santé représentaient 56,7 % de la variance totale (CCI = 0,567). Après ajustement sur les effets temporels, les différences géographiques demeuraient significatives, avec des probabilités plus élevées de résistance à l'érythromycine au Sud-Kivu (OR ajusté = 1,93 ; IC à 95 % : 1,31–2,85 ; p = 0,001) et au Tanganyika (OR ajusté = 4,02 ; IC à 95 % : 1,89–9,27 ; p < 0,001). La RAM chez V. cholerae en RDC se caractérise par une résistance quasi universelle à plusieurs antibiotiques historiquement utilisés, tandis que la sensibilité à la doxycycline et à l'azithromycine demeure préservée. Le gradient géographique marqué de la résistance à l'érythromycine, l'importante hétérogénéité intraprovinciale et l'interaction significative Province × Sérotype soulignent la nécessité de renforcer la surveillance à l'échelle des zones de santé afin d'orienter les stratégies thérapeutiques contre le choléra adaptées aux réalités locales.***

***Mots-clés : Vibrio cholerae ; résistance aux antimicrobiens ; choléra ; République démocratique du Congo ; modèles à effets mixtes ; hétérogénéité spatiale.***

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## I. INTRODUCTION

Cholera remains one of the most devastating diarrheal diseases worldwide, with an estimated 1.3–4 million cases and 21,000–143,000 deaths annually [1]. In the Democratic Republic of the Congo (DRC), cholera is endemic in the Great Lakes provinces, where recurrent outbreaks occur in the context of armed conflicts, deteriorating health

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infrastructure, and the circulation of epidemic strains [2–4]. Antimicrobial therapy represents an important adjunctive component of cholera management, alongside oral rehydration therapy, by reducing the duration of illness and limiting community transmission [5]. However, the emergence and spread of antimicrobial resistance (AMR) among *Vibrio cholerae* threaten the effectiveness of first-line treatments and complicate outbreak response efforts [6].

In sub-Saharan Africa, AMR patterns among *V. cholerae* are characterised by widespread resistance to sulfonamides, chloramphenicol, and ampicillin, with recent emergence of resistance to fluoroquinolones and macrolides in some settings [2,7,8]. The selection and dissemination of resistant strains are facilitated by extensive and often unregulated antibiotic use during cholera control interventions, as well as by the spread of mobile genetic elements such as conjugative integrons [9,10]. Nevertheless, data describing the geographic and temporal distribution of AMR patterns remain scarce, particularly in the DRC, where surveillance systems are constrained by logistical challenges and insecurity [2].

Spatial heterogeneity of AMR represents a critical challenge for optimising cholera treatment strategies. Studies conducted in Nigeria, Kenya, and Angola have demonstrated that specific epidemic lineages carrying diverse resistance determinants circulate according to distinct geographic patterns [9,11]. In the DRC, available evidence is largely limited to single-centre case series or interprovincial comparisons, without detailed assessment of within-province variability [8]. Such approaches may overlook substantial differences between operational units located within the same province. Indeed, fine-scale spatial variation within provinces may provide more actionable information for treatment decision-making than broad provincial comparisons, as it reflects local dynamics of transmission, antibiotic exposure, and dissemination of resistant clones. Therefore, decentralized AMR surveillance at the health zone level may be essential for early detection of emerging resistance hotspots and for tailoring treatment recommendations to local epidemiological contexts.

Statistical modelling of AMR determinants in *V. cholerae* should account for the hierarchical structure of surveillance data, in which isolates are clustered within health zones that are themselves nested within provinces. Ignoring this structure may lead to underestimated standard errors and inflated statistical significance of associations [12]. Generalized linear mixed-effects models (GLMMs) provide an appropriate analytical framework to simultaneously incorporate fixed effects of covariates and random effects arising from spatial clustering, while allowing exploration of complex temporal patterns [13].

The objective of this study was to characterize the geographic heterogeneity of AMR profiles among *V. cholerae* O1 isolates collected from three cholera hotspot provinces in eastern DRC, North Kivu, South Kivu, and Tanganyika, and to identify associated determinants, with particular attention to within-province variability at the health zone level. By quantifying the relative contribution of differences between health zones and provinces, this study also aimed to identify the most relevant spatial scale for AMR surveillance and for informing context-specific therapeutic recommendations. The findings are expected to support evidence-based cholera treatment strategies and strengthen integrated AMR surveillance within cholera control programs.

## II. MATERIAL AND METHODS

### II.1. Study design and population

This repeated analytical cross-sectional study used microbiological surveillance data from the AMI-LABO North Kivu Provincial Public Health Laboratory (Laboratoire de Santé Publique du Nord-Kivu AMI-LABO) located in Goma, in North Kivu Province, Eastern DRC, covering the period from January 2007 to December 2023. The laboratory receives and analyses stool specimens collected from patients with acute severe diarrhea (defined as ≥ 3 lose stools within 24 hours) managed in healthcare facilities across Eastern DRC.

The analysis included *V. cholerae* O1 isolates collected from North Kivu, South Kivu, and Tanganyika provinces, which are recognized as major cholera hotspots in the DRC (Figure 1). Extracted data included bacteriological identification results and antimicrobial susceptibility testing (AST) results. Only isolates with complete antimicrobial susceptibility profiles were included. Negative cultures and isolates that were not preserved in the laboratory strain collection were excluded.

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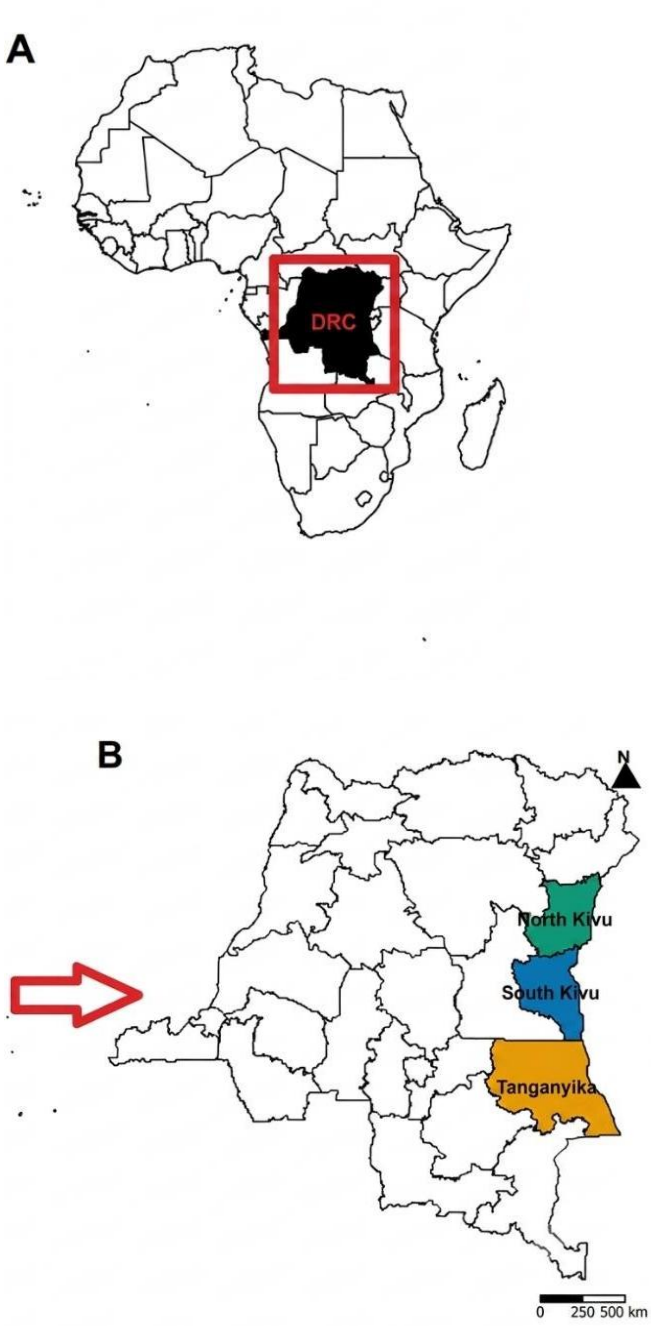



Figure 1. Geographic location of the Democratic Republic of the Congo (DRC) within Africa (Panel A) and study provinces within the DRC (Panel B). Panel A shows the DRC (shaded in black) on the African continent, with the red rectangle indicating the area enlarged in Panel B. Panel B displays the three study provinces: Tanganyika (orange), South Kivu (blue), and North Kivu (green). The scale bar represents 500 km. Maps were created using R version 4.3.3 using the ggplot2, sf, and patchwork packages.

### II.2. Bacteriological identification and antimicrobial susceptibility testing

Identification of *V. cholerae* O1 was performed according to the standard operating procedures of the AMI-LABO North Kivu Public Health Laboratory, as previously described in studies conducted by the laboratory [14]. Stool specimens or rectal swabs were transported to the laboratory using Cary–Blair transport medium and processed upon arrival.

Upon receipt, approximately 90% of each specimen was used for pre-enrichment in 1% alkaline peptone water for 6–8 hours at 37°C. Samples were subsequently subcultured onto selective thiosulphate citrate bile salts sucrose (TCBS) agar and incubated at 37°C for 18–24 hours. Presumptive identification was based on the observation of yellow colonies, characteristic of *V. cholerae* due to sucrose fermentation. Suspected colonies were confirmed using conventional biochemical testing (Kligler iron agar, urease, citrate, and SIM tests), the oxidase test (positive reaction), and agglutination with polyvalent O1 antisera, followed by serotyping using specific anti-Ogawa and anti-Inaba antisera.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. Inhibition zone diameters were converted into binary susceptibility categories according to the interpretative criteria applied by the AMI-LABO North Kivu Public Health Laboratory, based on Clinical and Laboratory Standards Institute (CLSI) recommendations and, more recently, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Ten antimicrobial agents were tested at the following concentrations: ampicillin (10 µg), tetracycline (30 µg), doxycycline (30 µg), chloramphenicol (30 µg), cotrimoxazole (25 µg), erythromycin (15 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), and azithromycin (15 µg). Inhibition zones were measured after 18–24 hours of incubation at 37°C. A reference strain was used for quality control of antimicrobial susceptibility testing.

### II.3. Variables

Resistance to each antimicrobial agent was coded as a binary variable (0 = susceptible, 1 = resistant). The multidrug resistance (MDR) profile was defined as simultaneous resistance to at least three antimicrobial agents. The number of resistant antimicrobial agents (Nb_R) was calculated on a scale ranging from 0 to 10.

Sociodemographic variables included age (in years), sex (male/female), province (North Kivu, South Kivu, and Tanganyika), and health zone (hierarchical clustering variable). Serotype information (Ogawa, Inaba, Hikojima, and undefined) was extracted from the bacteriological identification results. The year of specimen collection was derived from the sampling date, with management of multiple data formats (French text format and numerical Excel format).

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### II.4. Analytical strategy

The analysis consisted of four sequential steps. First, patient characteristics and antimicrobial resistance profiles were described using descriptive statistics and proportions with exact 95% confidence intervals (CIs) calculated using the Clopper–Pearson method [15]. Second, comparisons between provinces were performed using the $\chi^2$ test or analysis of variance (ANOVA), depending on the nature of the variables. Third, two complementary multivariable models were developed to address distinct analytical objectives.

Using data from the entire study period (2007–2023), a generalised linear mixed-effects model (GLMM) with a logit link function was fitted to assess erythromycin resistance. Isolates were considered clustered within health zones, with a random intercept included to account for the hierarchical structure of the data [12]. This model primarily aimed to quantify between-health-zone heterogeneity and to determine whether the association between serotype and erythromycin resistance differed across provinces.

Fixed effects included province (reference category: North Kivu), centred year of specimen collection, age, sex (reference category: male), and serotype (reference category: Ogawa). The Province × Serotype interaction was assessed using a likelihood ratio test.

To explore potential non-linear associations between calendar year and erythromycin resistance, a generalised additive model (GAM) and a generalised additive mixed model (GAMM) were additionally fitted. These analyses were considered exploratory, and model performance was compared with that of the primary GLMM.

In parallel, Firth's penalised logistic regression was applied to the 2021–2022 sub-period, corresponding to the only time window during which all three provinces were simultaneously represented. The primary objective of this model was to estimate the independent effect of province on erythromycin resistance after controlling for temporal confounding arising from differences in sampling periods between provinces.

Firth's penalisation approach was selected because of the presence of quasi-complete separation and low-frequency categories that could lead to biased estimates using conventional maximum likelihood estimation [16,17]. Variables included in the model were province, year, age, sex, and serotype.

Spatial heterogeneity was quantified from the GLMM using the intraclass correlation coefficient (ICC) [18,19]. Sensitivity analyses were conducted by comparing results from imputed datasets with those obtained from complete-case analyses, and by excluding South Kivu to assess the robustness of the findings. For antimicrobial agents showing complete separation, only descriptive results were reported.

### II.5. Model diagnostic and validation

Collinearity between explanatory variables was assessed using the variance inflation factor (VIF) calculated from the equivalent additive generalised linear model (GLM), with a tolerance threshold set at 5 [20]. The goodness-of-fit of the logistic regression models was evaluated using Nagelkerke's pseudo-$R^2$, the area under the receiver operating characteristic curve (AUC; c-statistic), and the Hosmer–Lemeshow goodness-of-fit test [21]. Convergence of mixed-effects models was assessed by examining the maximum gradient and the eigenvalues of the Hessian matrix [22]. Pearson residuals were examined to identify potential outliers.

### II.6. Missing data and imputation

Among the 25,421 stool specimens collected from the three provinces, missing data mainly concerned sociodemographic variables: age (1,355 missing values; 5.33%), sex (394 missing values; 1.55%), and health zone (7 missing values; 0.03%). The specimen collection date had 794 missing values (3.12%), some of which corresponded to invalid entries (e.g., health zone names entered in the date field). These records were retained, with the year variable coded as missing after data conversion.

Missing values in antimicrobial susceptibility variables (21,745 missing values; 85.54% for each antimicrobial agent) corresponded to negative cultures and were handled through exclusion. Only isolates with confirmed *V. cholerae* O1 growth were included in the analysis (n = 3,676).

For sociodemographic variables, simple imputation using the median (age and year) or the mode (sex and serotype), stratified by province, was performed. The proportion of imputed values was below 15% for each variable [23]. Seven records with residual missing values for health zone were excluded, resulting in a final analytical sample of 3,674 isolates.

A complete-case sensitivity analysis (n = 3,674; 0.05% loss of observations) yielded identical odds ratios to those obtained from the imputed dataset, confirming the robustness of the imputation approach.

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### II.7. Software and statistical significance threshold

All analyses were performed using R version 4.3.3 [24]. The following packages were used: *lme4* for GLMMs, *mgcv* for GAMs, *logistf* for Firth's penalised logistic regression, *car* for VIF estimation, *Resource Selection* for the Hosmer–Lemeshow test, and *ggplot2* for data visualisation. Statistical significance was defined as a two-sided *p*-value <0.05. Ninety-five per cent confidence intervals (95% CIs) were calculated using the Wald method for GLMM estimates.

### II.8. Ethical considerations

This study was a secondary analysis of routinely collected cholera surveillance data (2007– 2023) from the National Cholera Elimination and Diarrhoeal Disease Control Programme (PNECHOL-MD), the National Health Information System (SNIS), and the AMI-LABO North Kivu Provincial Public Health Laboratory in Goma, Democratic Republic of the Congo. The dataset included microbiological records from North Kivu, South Kivu, and Tanganyika, three cholera hotspot provinces identified in the Multisectoral Strategic Cholera Elimination Plan (PMSEC 2023–2027).

No direct patient contact or additional interventions were performed. Individual informed consent was not required for this secondary analysis of anonymised surveillance data. Personal identifiers were removed, and data were aggregated at health zone level to ensure confidentiality. Data use was authorised by the Ministry of Public Health of the DRC through PNECHOL-MD and the Provincial Health Divisions. Ethical approval was obtained from the Medical Ethics Committee of the University of Goma (Reference: UNIGOM/CEM/006/2022). The study complied with national regulations, the Declaration of Helsinki, and WHO guidance for research using routine surveillance data.

## III. RESULTS

### III.1. Patient characteristics

Among the 25,421 stool specimens collected from the three provinces during the study period, 3,676 (14.5%; 95% CI: 14.0–14.9) yielded confirmed *Vibrio cholerae* O1 isolates with available antimicrobial susceptibility testing results and were therefore included in the microbiological resistance analysis. These isolates comprised 2,256 (11.6%; 95% CI: 11.2–12.1) from North Kivu, 1,287 (25.9%; 95% CI: 24.7–27.1) from South Kivu, and 133 (13.0%; 95% CI: 11.0–15.2) from Tanganyika.

Among the 3,674 isolates retained for analysis after excluding two records with missing health zone information, 2,254 (61.4%) originated from North Kivu, 1,287 (35.0%) from South Kivu, and 133 (3.6%) from Tanganyika. The median age was 10.0 years (IQR: 4.0– 24.0), with a similar age distribution across provinces. The proportion of female patients was 58.1% in North Kivu, 48.4% in South Kivu, and 48.9% in Tanganyika.

The Ogawa serotype predominated in South Kivu (89.0%) and Tanganyika (45.9%), whereas the Inaba serotype was more frequent in North Kivu (41.4%). The study period covered 2007–2023, with a substantial concentration of samples collected during 2021– 2022 (Table 1).

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**Table 1. Sociodemographic and microbiological characteristics of *Vibrio cholerae* O1 isolates by province**

| Variable | North Kivu | South Kivu | Tanganyika |
|---|---|---|---|
| Total, n (%) | 2,254 (61.4) | 1,287 (35.0) | 133 (3.6) |
| Age, years (median [IQR]) | 10 (4–23) | 12 (5–24) | 17.5 (8–30) |
| Female sex, n (%) | 1,309/2,254 (58.1) | 623/1,287 (48.4) | 65/133 (48.9) |
| Serotype Ogawa, n (%) | 1,244/2,254 (55.2) | 1,146/1,287 (89.0) | 76/133 (57.1) |
| Serotype Inaba, n (%) | 933/2,254 (41.4) | 62/1,287 (4.8) | 57/133 (42.9) |
| Serotype Hikojima, n (%) | 67/2,254 (3.0) | 47/1,287 (3.7) | 0/133 (0) |
| *V. cholerae* serotype not defined,n (%) | 10/2,254 (0.4) | 32/1,287 (2.5) | 0/133 (0) |

***Note:*** *Values are presented as n, n (%), or median (interquartile range [IQR]), as appropriate. Percentages were calculated using the total number of isolates from each province as the denominator. IQR = interquartile range.*

### III.2. Antimicrobial resistance profiles

Resistance was nearly universal to ampicillin (99.8%), chloramphenicol (100%), cotrimoxazole (100%), and nalidixic acid (99.9%) across all provinces (Table 2). Conversely, no resistance was detected to tetracycline, doxycycline, norfloxacin, or azithromycin in any of the three provinces. Ciprofloxacin resistance was observed in only one isolate (0.03%, North Kivu).

**Table 2. Antimicrobial resistance of *Vibrio cholerae* isolates by province**

| Antibiotic | North Kivu, n = 2,254 % (95% CI) | South Kivu, n = 1,287 % (95% CI) | Tanganyika, n = 133 % (95% CI) |
|---|---|---|---|
| Ampicillin | 99.7 (99.4–99.9) | 99.9 (99.6–100) | 100 (97.3–100) |
| Chloramphenicol | 100 (99.8–100) | 100 (99.7–100) | 100 (97.3–100) |
| Cotrimoxazole | 100 (99.8–100) | 100 (99.7–100) | 100 (97.3–100) |
| Erythromycin | 28.2 (26.3–30.1) | 65.4 (62.8–68.0) | 86.5 (79.5–91.8) |
| Nalidixic acid | 100 (99.8–100) | 100 (99.7–100) | 100 (97.3–100) |
| Ciprofloxacin | 0 (0–0.2) | 0 (0–0.3) | 0 (0–2.7) |
| Azithromycin | 0 (0–0.2) | 0 (0–0.3) | 0 (0–2.7) |
| Doxycycline | 0 (0–0.2) | 0 (0–0.3) | 0 (0–2.7) |

***Note*****:** *Values are presented as percentages of resistant isolates with exact 95% confidence intervals calculated using the Clopper–Pearson method. Resistance was determined based on antimicrobial susceptibility testing results. Percentages were calculated using the number of isolates tested for each antimicrobial agent within each province as the denominator. Antimicrobial agents showing no resistance across all three provinces (tetracycline, doxycycline, norfloxacin, and azithromycin) were not included in the table. CI = confidence interval.*

The only marked geographic heterogeneity was observed for erythromycin resistance. Over the entire study period, erythromycin resistance increased progressively from North Kivu (28.2%; 95% CI: 26.3–30.1) to South Kivu (65.4%; 95% CI: 62.8–68.0) and Tanganyika (86.5%; 95% CI: 79.5–91.8) (Figure 2). This north-to-south gradient was statistically significant ($p < 0.001$).

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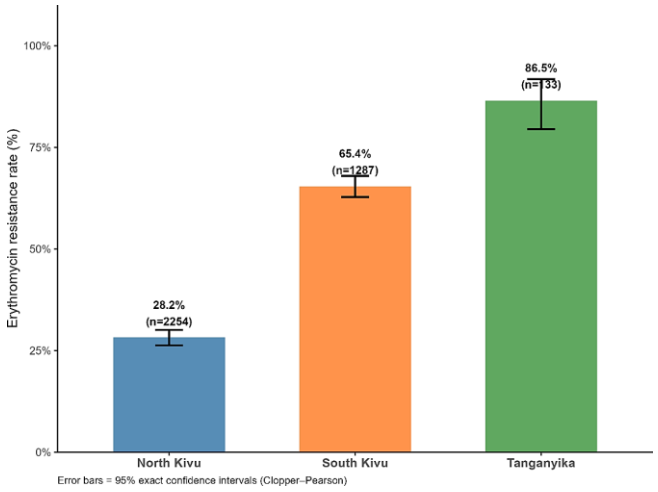



Figure 2. Erythromycin resistance rates by province. The wide confidence interval observed for Tanganyika (79.5–91.8%) reflects the smaller sample size (n = 133).

During the 2021–2022 sub-period, the distribution of erythromycin-susceptible and resistant isolates differed across provinces. The proportion of resistant isolates increased from 51.0% in North Kivu (379 susceptible vs 394 resistant isolates), to 68.7% in South Kivu (381 vs 837), and 86.5% in Tanganyika (18 vs 115). Serotype distribution varied across provinces, with Ogawa predominating in all three settings (North Kivu: 702 isolates; South Kivu: 1,099 isolates; Tanganyika: 76 isolates). The Inaba serotype was more frequent in Tanganyika (57 isolates) than in North Kivu (32 isolates) and South Kivu (46 isolates), whereas Hikojima and non-defined *V. cholerae* serotypes were not detected in Tanganyika.

Regarding erythromycin resistance, Ogawa accounted for the largest number of resistant isolates (1,172 resistant vs 705 susceptible isolates), followed by Inaba (86 vs 49) and Hikojima (51 vs 24). All isolates classified as non-defined *V. cholerae* serotypes were resistant (37 resistant vs 0 susceptible isolates).

Analysis at the health zone level revealed even greater within-province heterogeneity. In North Kivu, erythromycin resistance rates ranged from 0% (Kibua, *n* = 21) to 86.2% (Katoyi, *n* = 29). In South Kivu, variability was particularly pronounced, ranging from 8.6% (Uvira, *n* = 93) to 97.9% (Kalehe, *n* = 189). In Tanganyika, the five assessed health zones showed consistently high but heterogeneous resistance rates, ranging from 66.7% (Kongolo, *n* = 3) to 100% (Ankoro, *n* = 7; Kalemie, *n* = 58; Kansimba, *n* = 3).

The health zone-level intraclass correlation coefficient (ICC) estimated from the mixed- effects model was 0.567 (between-health-zone variance = 4.313; standard deviation = 2.077), indicating substantial clustering of erythromycin resistance at the health zone level and marked heterogeneity between health zones.

### III.3. Multivariable modelling

#### III.3.1. Generalised linear mixed-effects model: hierarchical structure and Province × Serotype interaction

A GLMM including the Province × Serotype interaction showed a significantly better fit than the corresponding additive model (likelihood ratio test [LRT]: $\chi^2 = 36.66$, degrees of freedom [df] = 4, $p = 2.12 \times 10^{-7}$; ΔAIC = 28.7) and was therefore retained as the primary model (Table 3).

**Table 3. Multivariable logistic regression model of factors associated with erythromycin resistance (GLMM, 2007–2023)**

| Variable | Adjusted OR (95% CI) | *p*-value |
|---|---|---|
| Intercept | 0.13 (0.05–0.33) | <0.001 |
| Province: South Kivu | 5.07 (1.80–14.32) | 0.002 |
| Province: Tanganyika | 192.43 (19.71–1879.15) | <0.001 |
| Serotype: Inaba | 1.78 (1.39–2.27) | <0.001 |
| Serotype: Hikojima | 2.00 (1.14–3.51) | 0.016 |
| Year (centred) | 1.02 (0.99–1.04) | 0.202 |
| Age (per year) | 1.00 (0.99–1.00) | 0.042 |
| Female sex | 0.73 (0.62–0.85) | <0.001 |
| Interaction: South Kivu × Inaba | 0.13 (0.06–0.28) | <0.001 |
| Interaction: Tanganyika × Inaba | 0.30 (0.09–0.99) | 0.047 |
| Interaction: South Kivu × Hikojima | 0.30 (0.13–0.72) | 0.006 |
| Interaction term (other) | 0.31 (0.02–6.27) | 0.442 |

***Note:*** *The interaction model was selected as the primary model based on the likelihood ratio test (LRT: $\chi^2 = 36.66$; df = 4; p = 2.12 × 10⁻⁷) and the difference in Akaike information criterion (ΔAIC = 28.7). Two interaction terms could not be estimated because of absent combinations in the dataset (Tanganyika × Hikojima and Tanganyika × non-defined* V. cholerae*). Reference categories were North Kivu (province), Ogawa (serotype), and male sex. Results are presented as adjusted odds ratios (aORs) with 95% confidence intervals and p-values from the multivariable logistic regression model. Continuous variables were modelled as specified (age per year and centred year). ORs and confidence intervals were rounded to two decimal places. CI = confidence interval.*

After adjustment for centred year of specimen collection, age, sex, and serotype, the odds of erythromycin resistance were significantly higher among isolates from South Kivu (adjusted OR [aOR] = 5.07; 95% CI: 1.80–14.32; $p = 0.002$) and Tanganyika (aOR = 192.43; 95% CI: 19.70–1879.23; $p < 0.001$) compared with North Kivu.

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Increasing age was associated with a marginal reduction in the odds of erythromycin resistance (aOR = 0.995 per year; 95% CI: 0.990–1.000; $p$ = 0.037), while female sex was associated with lower odds of resistance compared with male sex (aOR = 0.73; 95% CI: 0.61–0.85; $p < 0.001$).

Among isolates from North Kivu, which served as the reference category for the interaction analysis, the Inaba serotype was associated with higher odds of erythromycin resistance compared with Ogawa (OR = 1.78; 95% CI: 1.39–2.27; $p < 0.001$). Positive associations were also observed for Hikojima (OR = 2.00; 95% CI: 1.14–3.51; $p$ = 0.016) and non- defined *V. cholerae* serotypes (OR = 48.73; 95% CI: 5.23–453.72; $p < 0.001$).

The effect of serotype differed significantly according to province. Compared with North Kivu, the effect of the Inaba serotype was attenuated in South Kivu (interaction OR = 0.13; 95% CI: 0.06–0.28; $p < 0.001$) and Tanganyika (interaction OR = 0.30; 95% CI: 0.09–0.99; $p$ = 0.047). A significant interaction was also observed between South Kivu and the Hikojima serotype (interaction OR = 0.30; 95% CI: 0.13–0.72; $p$ = 0.006), whereas the South Kivu × non-defined *V. cholerae* interaction was not statistically significant (interaction OR = 0.31; 95% CI: 0.01–6.27; $p$ = 0.442). Interactions involving Tanganyika × Hikojima and Tanganyika × non-defined *V. cholerae* could not be estimated because of the absence of observations in these categories.

A linear mixed-effects model assessing the total number of antimicrobial resistances (Nb_R) produced consistent findings. Compared with North Kivu, isolates from Tanganyika had, on average, 0.62 additional resistant antimicrobial agents (t = 4.37; $p < 0.001$), compared with 0.15 additional resistances in South Kivu (t = 1.97; $p$ = 0.049) (Figure 3).

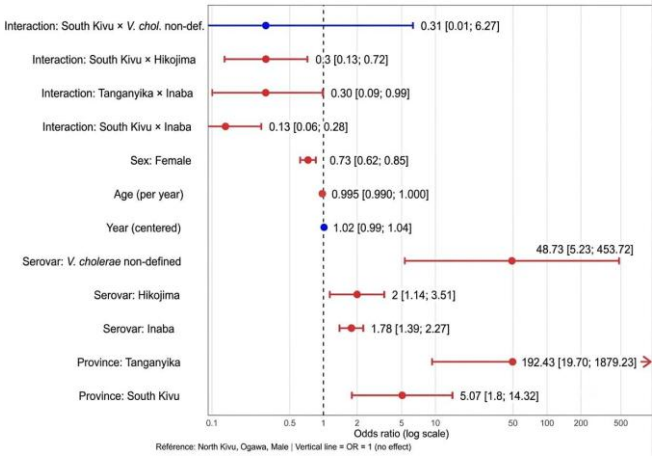



Figure 3. Adjusted odds ratios of erythromycin resistance from the primary generalised linear mixed-effects model including Province × Serotype interaction. The model included centred year of specimen collection, age, sex, serotype, province, and a random intercept for health zone. Error bars represent 95% Wald confidence intervals. The vertical dashed line indicates no association (OR = 1). Reference categories were North Kivu (province), Ogawa (serotype), and male sex.

### III.3.2. Firth penalized logistic regression: provincial effect after controlling for temporal confounding

To estimate the independent effect of province while accounting for differences in sampling periods, Firth penalised logistic regression was fitted using the common 2021–2022 period ($n$ = 2,124), during which all three provinces were simultaneously represented (Table 4).

**Table 4. Firth penalized logistic regression model of factors associated with erythromycin resistance (n = 2,124)**

| Variable | Adjusted OR (95% CI) | *p*-value |
|---|---|---|
| **Province** | | |
| North Kivu | Reference | – |
| South Kivu | 1.93 (1.31–2.85) | 0.001 |
| Tanganyika | 4.02 (1.89–9.27) | <0.001 |
| Age (years) | 0.99 (0.98–1.00) | 0.083 |
| **Sex** | | |
| Male | Reference | – |
| Female | 1.29 (0.89–1.88) | 0.185 |
| **Serotype** | | |
| Ogawa | Reference | – |
| Inaba | 0.20 (0.12–0.35) | <0.001 |
| Hikojima† | 2.00 (1.14–3.51) | 0.016 |
| Non-defined *V. cholerae*† | 48.73 (5.23–453.72) | <0.001 |

***Notes:***

- *The model assessed whether residence in South Kivu or Tanganyika remained associated with erythromycin resistance after controlling for temporal variation and covariates (age, sex, and serotype).*
- *Firth penalised logistic regression was fitted on the common 2021–2022 period (*n *= 2,124) to control for temporal confounding caused by differences in sampling distribution across provinces (likelihood ratio test: $\chi^2 = 1959.8$; df = 8; $p < 0.001$).*
- *The model included centred year of collection to account for annual fluctuations in resistance. The OR for year is not displayed because its estimate was highly unstable over the two years (OR = 17,581; 95% CI: 2,417–2,072,175).*
- *Confidence intervals were calculated using penalised profile likelihood.*
- *OR = odds ratio; CI = confidence interval. † Estimates for Hikojima and non-defined V. cholerae were derived from the full-period GLMM (2007– 2023), as their numbers in the 2021–2022 subset were too small to provide stable Firth regression estimates.*

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After adjustment for year, age, sex, and serotype, the odds of erythromycin resistance remained significantly higher in South Kivu (aOR = 1.93; 95% CI: 1.31–2.85; $p = 0.001$) and Tanganyika (aOR = 4.02; 95% CI: 1.89–9.27; $p < 0.001$) compared with North Kivu.

The Inaba serotype was associated with significantly lower odds of erythromycin resistance (aOR = 0.20; 95% CI: 0.12–0.35; $p < 0.001$), whereas the Hikojima serotype was associated with higher odds of resistance (aOR = 16.68; 95% CI: 1.57–3480.44; $p = 0.009$). Associations with age ($p = 0.083$), sex ($p = 0.185$), and non-defined *V. cholerae* serotypes (*$p = 0.074$) were not statistically significant.*

### III.4. Sensitivity and robustness analyses

A complete-case sensitivity analysis ($n = 3,674$; 0.05% loss due to two missing health zone values) yielded identical odds ratios to those obtained from the imputed dataset, confirming the robustness of the imputation approach (Table 5).

**Table 5. Sensitivity analysis: imputed dataset versus complete-case analysis**

| Variable | Imputed dataset aOR (95% CI) | Complete-case aOR (95% CI) |
|---|---|---|
| Intercept | 0.13 (0.05–0.33) | 0.13 (0.05–0.33) |
| South Kivu vs North Kivu | 5.07 (1.80–14.32) | 5.07 (1.80–14.32) |
| Tanganyika vs North Kivu | 192.43 (19.70–1879.23) | 192.43 (19.70–1879.23) |
| Inaba vs Ogawa | 1.78 (1.39–2.27) | 1.78 (1.39–2.27) |
| Hikojima vs Ogawa | 2.00 (1.14–3.51) | 2.00 (1.14–3.51) |
| Non-defined V. cholerae vs Ogawa | 48.73 (5.23–453.72) | 48.73 (5.23–453.72) |
| Year (centred) | 1.02 (0.99–1.04) | 1.02 (0.99–1.04) |
| Age (years) | 0.995 (0.99–1.00) | 0.995 (0.99–1.00) |
| Female vs male | 0.73 (0.61–0.85) | 0.73 (0.61–0.85) |
| South Kivu × Inaba | 0.13 (0.06–0.28) | 0.13 (0.06–0.28) |
| Tanganyika × Inaba | 0.30 (0.09–0.99) | 0.30 (0.09–0.99) |
| South Kivu × Hikojima | 0.30 (0.13–0.72) | 0.30 (0.13–0.72) |
| South Kivu × non-defined V. cholerae | 0.31 (0.02–6.27) | 0.31 (0.02–6.27) |

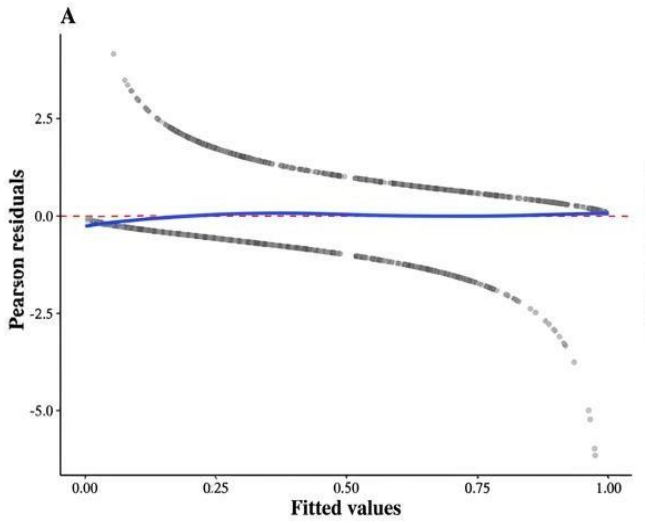
***Note:*** *OR estimates and confidence intervals from the imputed and complete-case approaches were identical to three decimal places. Both analyses included 3,674 observations; only two observations (0.05%) were excluded from the complete-case analysis because of missing health zone information.*

Sensitivity analysis excluding South Kivu ($n = 2,387$; 35.0% reduction in sample size) confirmed the stability of the main findings. The association between Tanganyika and erythromycin resistance remained strong and statistically significant (OR = 94.08; 95% CI: 13.81–640.97; $p < 0.001$). Female sex remained associated with lower odds of resistance (OR = 0.54; 95% CI: 0.44–0.66; $p < 0.001$), whereas the Inaba serotype remained associated with increased odds of resistance (OR = 1.35; 95% CI: 1.06–1.72; $p = 0.015$).

In this restricted analysis, the year of specimen collection became statistically significant (OR = 0.96; 95% CI: 0.94–0.99; $p = 0.003$), suggesting a temporal association potentially influenced by the underlying provincial distribution of isolates in the full dataset.

### III.5. Model diagnostic (GLMM)

Collinearity among explanatory variables was low, with variance inflation factor (VIF) values below 1.5 for all variables included in the model, supporting their simultaneous inclusion. The Nagelkerke pseudo-$R^2$ was 0.0327, indicating a limited contribution of fixed-effect covariates to overall model variation. The area under the receiver operating characteristic curve (AUC) was 0.824 (95% CI: 0.811–0.837). The Hosmer–Lemeshow test applied to the GLMM was statistically significant ($\chi^2 = 73.27$, df = 8, $p < 0.001$), reflecting the known sensitivity of this test in large samples ($n = 3,674$) (Figure 4).




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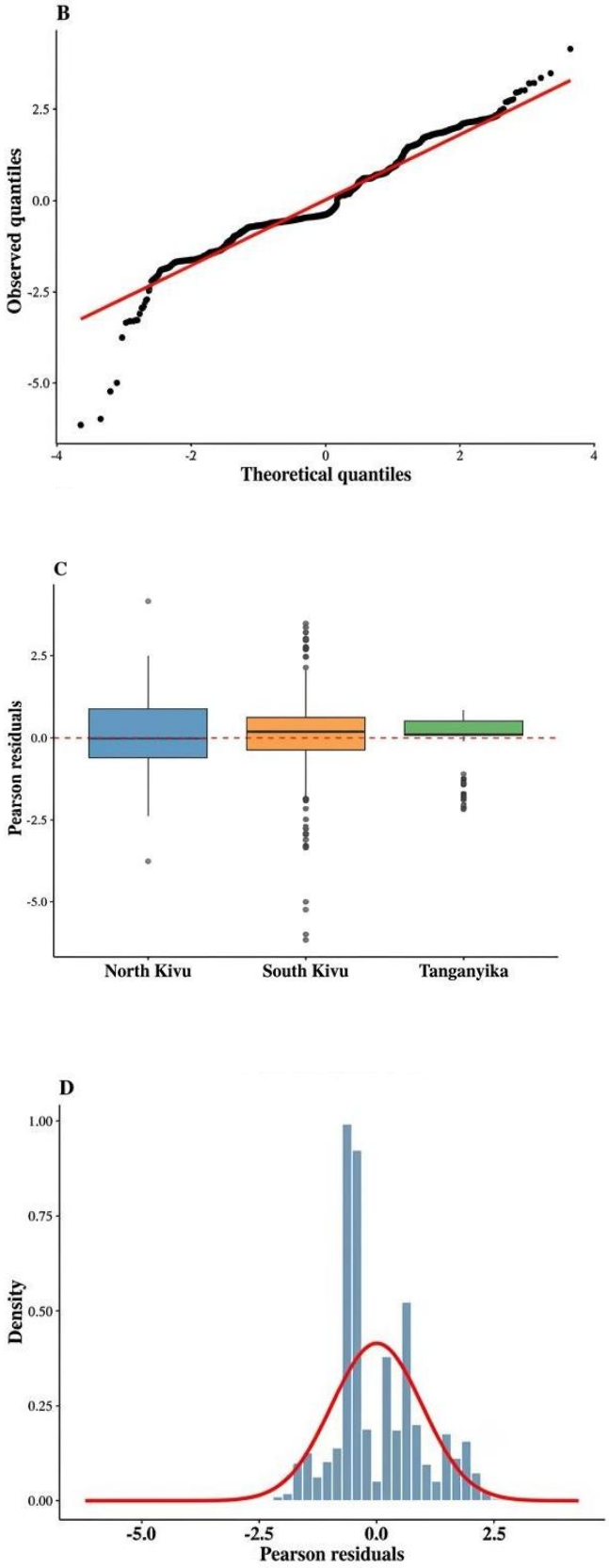



Figure 4. Residual diagnostics of the primary GLMM with Province × Serotype interaction. (A) Pearson residuals versus fitted values; (B) QQ plot of Pearson residuals; (C) distribution of Pearson residuals by province; (D) histogram of Pearson residuals with normal density curve overlay.

Diagnostics of the primary GLMM indicated no major violations of model assumptions (Figure 4). Pearson residuals versus fitted values showed no funnel-shaped pattern, and the LOESS curve remained approximately centred around zero (Figure 4A). The QQ plot demonstrated an approximately normal distribution of residuals around the median, with mild tail deviations compatible with binary outcome data (Figure 4B). Residuals were centred around zero across provinces, although greater dispersion was observed in South Kivu (Figure 4C). The residual histogram showed a unimodal distribution with slight right skewness, consistent with the observed clustering structure (ICC = 0.567) and unequal provincial sample distribution (Figure 4D).

## IV. DISCUSSION

This study is the first to simultaneously investigate inter- and intra-provincial heterogeneity of antimicrobial resistance (AMR) among *Vibrio cholerae* O1 isolates in the Democratic Republic of the Congo (DRC). We identified a resistance profile characterised by a classical trimodal pattern, with near-universal resistance to several historical antimicrobial agents, a marked geographic gradient for erythromycin resistance, and greater spatial variability at the health zone level than at the provincial level.

The near-complete resistance to ampicillin, chloramphenicol, and cotrimoxazole observed in our study is consistent with regional evidence from sub-Saharan Africa [7,8]. These three antimicrobial agents, historically used for cholera management in the DRC, have likely exerted substantial selective pressure favouring the expansion of resistant strains, potentially through the dissemination of mobile genetic elements, including SXT integrative conjugative elements [9,25,26]. Conversely, the complete absence of resistance to tetracycline, doxycycline, and azithromycin is reassuring and confirms that these antimicrobial agents remain effective therapeutic options in the DRC, in contrast with some Asian settings where azithromycin resistance has increasingly been reported [25]. This favourable susceptibility profile should be preserved through strengthened antimicrobial stewardship, including avoidance of unnecessary or prophylactic use of macrolides.

The erythromycin resistance gradient represents the main finding of this study. Several mechanisms may contribute to this geographic pattern. First, South Kivu and Tanganyika, which are geographically more distant from centralised surveillance structures and antibiotic supply networks, may experience different patterns of antimicrobial access and use, potentially generating distinct selective pressures favouring resistance emergence [27]. In addition, the high prevalence of self-medication practices in many low-resource settings may

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further contribute to inappropriate antimicrobial exposure and increase the risk of resistance selection.

Second, the geographic distribution of erythromycin resistance may reflect the circulation of specific epidemic clones carrying resistance determinants, such as *erm* or *mef* genes, in particular ecological settings [6]. Third, the significant Province × Serotype interaction observed in our study suggests geographic variation in *V. cholerae* population structure. In North Kivu, the Inaba serotype was associated with increased odds of erythromycin resistance; however, this association was attenuated or reversed in South Kivu and Tanganyika. This pattern may reflect differences in the distribution of resistant clones across provinces, including the circulation of highly resistant strains classified as non- defined serotypes, which could mask the independent effect of Inaba.

An important contribution of this study is the demonstration that spatial variability within provinces may be more informative than provincial comparisons alone. Although differences between provinces were substantial, the high intraclass correlation coefficient observed at the health zone level indicates considerable heterogeneity between operational units within the same province. These findings highlight that AMR surveillance strategies based solely on provincial averages may overlook important local transmission and resistance patterns. A decentralised surveillance approach at the health zone level may therefore provide more relevant information for adapting cholera treatment policies to local epidemiological contexts.

The marked imbalance in isolate numbers between provinces (2,254 isolates from North Kivu compared with 133 from Tanganyika) should not be interpreted as reflecting lower cholera incidence in Tanganyika, a recognised endemic hotspot with several thousand reported cases annually. Rather, this difference likely reflects limitations in microbiological detection and specimen referral pathways. Until 2025, Tanganyika lacked an on-site cholera reference laboratory, and specimens often required long-distance transport to Kalemie, with logistical constraints related to transport duration, specimen preservation, and cold-chain maintenance potentially limiting systematic sample collection. In contrast, North Kivu benefited from continuous laboratory capacity through AMI-LABO in Goma since 2007.

This potential selection bias may have resulted in under-representation of moderate cases from Tanganyika, while samples reaching the reference laboratory may have disproportionately represented severe or recurrent cases with greater prior antimicrobial exposure. Consequently, the high erythromycin resistance rate observed in Tanganyika (86.5%) should be interpreted cautiously. The establishment of dedicated cholera laboratory capacity in Tanganyika in 2025 represents an important opportunity to improve representativeness and strengthen local AMR surveillance.

The difference in the magnitude of the Tanganyika effect between the GLMM (OR = 192) and the Firth regression restricted to the 2021–2022 period (OR = 4.02) indicates that part of the observed difference was related to temporal sampling patterns, as Tanganyika isolates were predominantly collected during more recent years. However, the persistence of a statistically significant provincial effect after controlling for time suggests that the geographic gradient cannot be fully explained by a temporal artefact. Although more conservative, the estimate obtained from the Firth model (OR = 4.02) likely represents the most robust measure of the provincial effect because it accounts for the common observation period and reduces instability related to sparse data and quasi-complete separation [28–30].

The substantial intra-provincial heterogeneity, exceeding the variability observed between provinces, represents an important methodological and operational finding. The health zone-level ICC of 0.567 indicates considerable unexplained spatial variation after adjustment for measured covariates. A uniform provincial-level antimicrobial prescription policy may therefore be insufficient in settings where erythromycin resistance varies from 0% to 97.9% between health zones within the same province. This observation is consistent with findings from Quilici et al. [11] in Nigeria, where atypical multidrug-resistant El Tor strains displayed highly localised geographic circulation patterns.

The selection of the GLMM with Province × Serotype interaction as the primary analytical model was based on three considerations. First, the hierarchical structure of the data required the inclusion of a random effect for health zone to account for clustering and avoid underestimation of uncertainty due to correlated observations [12]. Second, the interaction term was highly significant and biologically interpretable, suggesting that the association between serotype and erythromycin resistance differed according to geographic context. Third, the GLMM framework provided directly interpretable effect estimates suitable for epidemiological interpretation. Generalised additive models (GAM) and generalised additive mixed models (GAMM) were explored in sensitivity analyses but were not retained as primary models. The GAM converged with warnings and produced unstable parameter estimates, including inconsistent coefficient directions for Inaba and implausibly large estimates for Hikojima and non-defined *V. cholerae*. The GAMM converged using penalised quasi-likelihood (PQL), but estimates were unstable. Furthermore, direct comparison of AIC values with the GLMM was not

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appropriate because the models relied on different estimation procedures (REML and PQL versus maximum likelihood with Laplace approximation).

This study has several limitations. First, its retrospective design limited access to important potential confounders, including previous antimicrobial exposure, vaccination status, and travel history, which could have contributed to the observed associations. Second, the unequal distribution of isolates across provinces and time periods—North Kivu contributing data from 2007 to 2023, whereas South Kivu and Tanganyika were more heavily represented during 2021–2022—limits the ability to fully disentangle geographic effects from temporal trends. Therefore, part of the observed erythromycin resistance gradient may reflect changes over time in addition to genuine spatial heterogeneity. Nevertheless, the persistence of a provincial effect after adjustment for a common observation period supports the existence of a genuine geographic component.

The absence of molecular data prevented identification of the genetic mechanisms underlying the observed geographic gradient and limited our ability to distinguish local selection from clonal dissemination. Simple imputation of missing data, although justified by the low proportion of missing values (<15%), may have underestimated uncertainty around parameter estimates. However, complete-case sensitivity analyses produced identical odds ratios to those obtained from the imputed dataset, supporting the robustness of the findings. Finally, the generalisability of our results to the entire DRC is limited by the absence of provinces from central regions and Greater Kasai, and by the lack of environmental and ecological covariates at health zone level that could help explain the marked intra-provincial heterogeneity observed.

Despite these limitations, this study provides the first hierarchical quantification of spatial heterogeneity in *V. cholerae* AMR in the DRC and identifies the health zone as the most relevant operational level for AMR surveillance and response.

The practical implications are immediate. Doxycycline and azithromycin remain the most appropriate therapeutic options among the tested agents in the three provinces. Erythromycin use should be reconsidered in South Kivu and Tanganyika, where resistance levels were consistently high, and susceptibility testing should be considered where its use is contemplated, particularly in North Kivu. Establishing prospective AMR surveillance at health zone level, integrated within existing cholera surveillance systems, is urgently needed to detect emerging resistance patterns and support locally adapted treatment strategies.

From an operational perspective, these findings support four priorities: (i) maintaining doxycycline and azithromycin as key therapeutic options in the three provinces, while continuing susceptibility monitoring; (ii) avoiding empirical reliance on erythromycin in South Kivu and Tanganyika; (iii) implementing decentralised AMR surveillance at health zone level, including systematic antimicrobial susceptibility testing where feasible; and (iv) strengthening diagnostic capacity in Tanganyika, which has recently established dedicated cholera laboratory capacity (2025).

## V. CONCLUSION

This study provides the first empirical evidence that AMR among *Vibrio cholerae* O1 in the Democratic Republic of the Congo follows a hierarchical spatial distribution, with greater variability occurring within provinces than between provinces. The significant Province × Serotype interaction indicates that *V. cholerae* population structures differ geographically, limiting the interpretation of province-level averages and supporting the use of mixed- effects modelling approaches.

The marked fine-scale spatial heterogeneity observed in this study highlights the need for AMR surveillance at the health zone level to guide more precise and locally adapted therapeutic decisions. Doxycycline and azithromycin remain the most reliable therapeutic options among the tested antimicrobials across the three provinces, whereas erythromycin should be avoided as an empirical option in areas with high resistance levels, particularly South Kivu and Tanganyika.

Integrating routine molecular characterisation into epidemiological surveillance would provide additional insight into the evolution and dissemination of resistance determinants and improve preparedness for the emergence of new resistant *V. cholerae* lineages.

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