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Original Article **Open Access**

Phenotypic characterization and antimicrobial susceptibility profiles of *Vibrio cholerae* isolates during the October 2022 and January 2023 outbreak in North-Kivu province, The Democratic Republic of Congo

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Abstract:

Background: Cholera is an infectious disease characterized by severe watery diarrhea, frequently occurring in outbreaks which affects many communities in the Democratic Republic of the Congo (DRC). At the end of October 2022, a cholera outbreak was declared in the camp of internally displaced people (IDP) of Kanyaruchinya, 20 kilometers north of Goma, the provincial capital of the North-Kivu province in DRC, as well as in other IDP camps and settlements around the city of Goma. The aim of this study was to phenotypically characterize *Vibrio cholerae* isolates associated with this outbreak, and to determinate their antimicrobial susceptibility profiles.
Methodology: Between October 31, 2022 and January 31, 2023, faecal swab samples were collected into Cary-Blair medium from 1604 and 538 patients with clinical signs of cholera at the Kanyaruchinya IDP, and IDPs camps and settlements around the city of Goma, respectively. After enrichment in 1% alkaline peptone water, the samples were cultured on thiosulphate-citrate-bile salt-sucrose (TCBS) agar for isolation and phenotypic characterization of *V. cholerae* O1 using conventional biochemical tests and serotyping technique. Antimicrobial susceptibility of selected isolates was peformed to a panel of 8 antibiotics by the disk diffusion method in accordance with EUCAST and CLSI guidelines.
Results: *Vibrio cholerae* was cultured from 807 samples (50.3%) of 1604 patients from the Kanyaruchinya IDP, and from 206 samples (38.3%) of 538 patients around the city of Goma (p<0.01). All the *V. cholerae* isolates from the Kanyaruchinya IDP (807/807, 100.0%) were serotyped as *V. cholerae* O1 Inaba whereas 136 (66.0%), 67 (32.5%), and 3 (1.5%) *V. cholerae* O1 isolates from around Goma were serotyped as *V. cholerae* O1 Ogawa, *V. cholerae* O1 Inaba, and Hikojima respectively. Antimicrobial susceptibility test on 174 and 62 isolates selected randomly from the 807 and 206 *V. cholerae* isolates from the Kanyaruchinya IDP camp, and from around the city of Goma respectively, showed that all the tested *V. cholerae* O1 isolates were resistant to polymyxin and cotrimoxazole, while being susceptible to tetracycline and azithromycin. All tested *V. cholerae* O1 isolates from Kanyaruchinya IDP camp displayed a unique antimicrobial susceptibility profile characterized by resistance to ampicillin, cotrimoxazole and chloramphenicol, and susceptiblility to ciprofloxacin, norfloxacin, azithromycin, tetracycline and doxycycline. Their counterparts from settlements around the city of Goma displayed a more variable antimicrobial susceptibility profile.
Conclusion: Our results suggest that a single *V. cholerae* O1 Inaba clone probably caused the cholera outbreak in the Kanyaruchinya IDP camp, whereas during the same period, several *V. cholerae* clones (Ogawa, Inaba and Hikojima) were associated with the cholera outbreak around the city of Goma.

Keywords : *Vibrio cholerae* O1, antimicrobial susceptibility, North-Kivu, DRC

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Caractérisation phénotypique et profils de sensibilité aux antimicrobiens des isolats de *Vibrio cholerae* lors de l'épidémie d'Octobre 2022 à Janvier 2023 dans la province du Nord-Kivu, République démocratique du Congo

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Résumé:

Contexte: Le choléra est une maladie infectieuse caractérisée par une diarrhée aqueuse sévère et se manifestant fréquemment par des épidémies qui affectent de nombreuses communautés en République démocratique du Congo (RDC). A la fin du mois d'Octobre 2022, une épidémie de choléra s'est déclarée dans le camp de déplacés internes (CDI) de Kanyaruchinya, situé à 20 kilomètres au nord de Goma, la capitale provinciale de la province du Nord-Kivu en RDC, ainsi que dans d'autres camps de déplacés et des bourgades autour de la ville de Goma. L'objectif de cette étude était la caractérisation phénotypique des isolats de *Vibrio cholerae* associés à cette épidémie et de déterminer leurs profils de sensibilité aux antimicrobiens.
Méthodologie: Entre le 31 Octobre 2022 et le 31 Janvier 2023, des écouvillons fécaux ont été recueillis dans un milieu Cary-Blair respectivement auprès de 1604 et 538 patients présentant des signes cliniques de choléra dans les camps de déplacés de Kanyaruchinya et les CDI les bourgades autour de la ville de Goma. Après enrichissement dans de l'eau peptonée alcaline à 1%, les échantillons ont été cultivés sur de la gélose thiosulfate-citrate-sel-bile-sucrose (TCBS) pour l'isolement et la caractérisation phénotypique de *V. cholerae O1*, en utilisant des tests biochimiques conventionnels et une technique de sérotypage. La sensibilité antimicrobienne des isolats sélectionnés a été évaluée pour un panel de 8 antibiotiques par la méthode de diffusion sur disque, conformément aux directives de l'EUCAST et du CLSI.
Résultats: *Vibrio cholerae* a été isolé à partir de 807 échantillons (50,3%) provenant de 1604 patients du CDI de Kanyaruchinya, et de 206 échantillons (38,3%) provenant de 538 patients autour de la ville de Goma (p<0,01). Tous les isolats *de V. cholerae* du CDI de Kanyaruchinya (807/807, 100,0%) ont été typés comme *V. cholerae O1* Inaba, tandis que 136 (66.0%), 67 (32.5%) et 3 (1.5%) isolats de *V. cholerae O1* des environs de Goma ont été typés comme *V. cholerae* O1 Ogawa, *V. cholerae* O1 Inaba et Hikojima. Les tests de susceptibilité aux antimicrobiens effectués sur 174 et 62 isolats sélectionnés au hasard parmi les 807 et 206 isolats de *V. cholerae* provenant respectivement du CDI de Kanyaruchinya et des environs de la ville de Goma ont montré que tous les isolats de *V. cholerae O1* testés étaient résistants à la polymyxine B et au cotrimoxazole, tout en étant susceptibles à la tétracycline et à l'azithromycine. Tous les isolats de *V. cholerae O1* testés dans le CDI de Kanyaruchinya présentaient un profil de sensibilité antimicrobienne unique, caractérisé par une résistance à l'ampicilline, au cotrimoxazole et au chloramphénicol, et une sensibilité à la ciprofloxacine, à la norfloxacine, à l'azithromycine, à la tétracycline et à la doxycycline. Leurs homologues des bourgades et CDI situés autour de la ville de Goma présentaient un profil de susceptibilité antimicrobienne plus variable.
Conclusion: Nos résultats suggèrent qu'un seul clone de *V. cholerae* O1 Inaba a probablement causé l'épidémie de choléra dans le CDI de Kanyaruchinya, alors qu'au cours de la même période, plusieurs clones de *V. cholerae* (Ogawa, Inaba et Hikojima) ont été associés à l'épidémie de choléra autour de la ville de Goma.

Mots-clés : *Vibrio cholerae* O1, susceptibilité aux antibiotiques, Nord-Kivu, North-Kivu, RDC

Introduction:

Cholera is an acute and life-threatening diarrheal disease caused by a comma shaped Gram-negative bacterium named *Vibrio cholerae* (1,2). The disease, which has been known for centuries, originated in Asia, and has evolved as pandemics (3). The current pandemic (7th), which started in 1961 is caused by *V. cholerae* serogroup O1 or O139, biovar El Tor (4), and has been characterized by large outbreaks in several developing countries (5).

It is estimated that up to near 3 million cases of people are affected annually by cholera, and that this resulted in 21,000 to 143,000 deaths worldwide in 2015 (6). Sub-Saharan Africa has become the leading part of the world with respect to cholera cases (7, 8) as highlighted by the recent large cholera outbreaks in Malawi (9) and in Mozambique (10). However recent outbreaks in Yemen (11) and Haiti (12) are a reminder of the cholera burden on other continents.

Despite the recent decrease in cholera cases worldwide, which has been lauded by the World Health Organization (13), The Democratic Republic of Congo (DRC) has continued to experience multiple cholera outbreaks, essentially in the eastern provinces of the country, a vast territory which has been plagued by civil unrest for the last thirty years resulting in millions of IDP, malnutrition, outbreaks of infectious diseases and a high number of deaths (14). The renewed fighting between the outcast M23 rebellion and the Congolese Armed Forces since April 2022 has aggravated the already volatile situation in the North-Kivu province, and resulted in massive displacements of people in the districts of Rutshuru, Nyiragongo, Masisi around the city of Goma, with the creation of a huge camps for internally displaced people (IDP) in Bulengo, Mugunga, and Kanyaruchinya.

The Kanyaruchinya IDP camp which

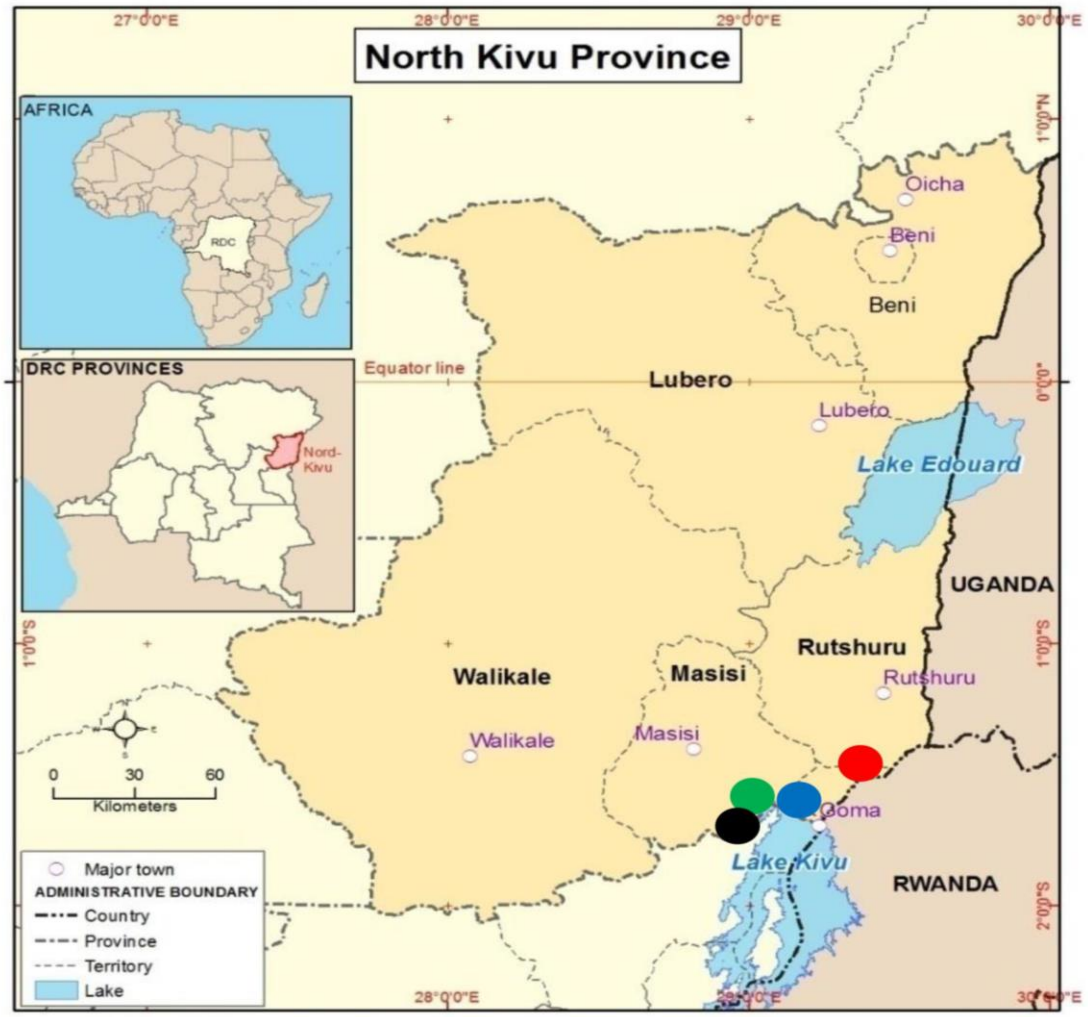
hosted 255,520 people at the end of October 2022 reported the first cases of severe watery diarrhea on October 31, 2022. This triggered the set-up of a cholera treatment center (CTC) for treatment of cases. A total of 3,917 cases were reported with 22 case fatalities. The cholera outbreak was declared as over on January 31 2023. During the same period, a spike in cases of severe watery diarrhea was reported in several settlements around the city Goma, including Bulengo and Mugunga IDP camps. Subsequent to this in suspected cholera cases in Kanyaruchinya IDP, an advanced laboratory for isolation and characterization of *V. cholerae* was set up in the preccint of the IDP camp in the aim of improving the management of this severe cholera outbreak, while the AMI-LABO reach back facility in Goma continued to process samples collected around the city of Goma.

This study describes the phenotypic characteristics and antimicrobial susceptibility profiles of *V. cholerae* O1 isolates from patients suspected with cholera in the Kanyaruchinya IDP camp and in the settlements around the city of Goma between October 31, 2022 and January 31, 2023.

Materials and method:

Study setting:

The districts of Rutshuru and Nyiragongo, which have been the epicentre of protracted civil war since 1996 are located in a range of 20 km to 80 km north of the North-Kivu provincial capital of Goma, which lies on the northern edge of Lake Kivu (Fig 1)



Red circle: Kanyaruchinya IDP; Blue circle: Buhimba settlement; Green circle: Mugunga IDP; Black circle: Bulengo IDP

Fig 1: Map of the North-Kivu province showing the cholera spots between October 31, 2022 and January 31, 2023

Ethical considerations:

Ethical approval for the study was granted by the Comité National d'Ethique de la Santé Publique (CNES) under the reference 6/BUR-CNES/NK/2023. A waiver for written informed consent was obtained from the same body after consultation with the North-Kivu Provincial Healthcare Division (Division Provinciale de la Santé), who both deemed an oral consent sufficient, given the dire circumstances created by the new wave of violence, and the magnitude of the cholera outbreak in the huge camp for IDPs, and around Goma.

The study complied with the World Health Organization (WHO) and international guidelines on investigation during outbreaks (https://apps.who.int/iris/bitstream/handle/10665/250580/9789241549837-eng.pdf). Personal identifiers were removed so that analyses of stored isolates were not traceable to individual patients. Each sample was labelled using a code referring to the date and location of sampling.

Study period, participants and sample collection:

From October 31, 2022 to January 31, 2023, a total of 3,917 patients meeting the clinical case definition of cholera (i. e. an acute watery diarrhea with or without vomiting in a patient with more than one year of age) were admitted in the ward of the cholera treatment center (CTC) of the Kanyaruchinya IDP camp in the North-Kivu province. None of the patients reported having taken antibiotics between symptoms onset and admission in the CTC ward of the Kanyaruchinya IDP camp. During the same period, 538 patients suspected of cholera in the settlements around the city of Goma were assessed for the presence of *V. cholerae* in their stools samples.

Rectal swab specimens were collected from a total of 2142 patients (1604 from the Kanyaruchinya IDP camp, and 538 from the settlements around the city of Goma) upon their arrival at the CTC or at the healthcare center, and before administration of antibiotics. The rectal swabs were put in Carry-Blair medium and immediately shipped to the laboratory for isolation of *V. cholerae*.

Culture isolation and identification of *V. cholerae*:

Laboratory testing was performed by trained personnel following the study protocol. Upon arrival at the laboratory, rectal swabs were incubated in 1% alkaline peptone water broth for 6-8 hours, and subsequently streaked onto thiosulfate-citrate-bile salt sucrose (TCBS) agar and incubated at 37°C for 16–24 hours. Large flattened yellow colonies with opaque centers and translucent peripheries were sub-cultured on nutrient alkaline agar plates overnight.

The colonies were further characterized by Gram-staining and light microscopic examination, and conventional biochemical tests such as oxidase, Kligler's iron agar for fermentation of carbohydrates, Voges-Proskauer and methyl red. Polyvalent O1, Ogawa and Inaba antisera (Becton Dickinson, Erembodegem, Belgium) were used for serotyping of the *V. cholerae* isolates, according to the manufacturer's recommendations.

Antimicrobial susceptibility testing:

Susceptibility of the *V. cholerae* O1 isolates to 8 antimicrobial agents (ampicillin, azithromycin, chloramphenicol, doxycycline, co-trimoxazole, ciprofloxacin, norfloxacin and tetracycline) was determined by the disk diffusion method according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as updated in January 2023, and the CLSI for antibiotics for which no EUCAST breakpoints were available. *Escherichia coli* ATCC 35218 was used as control for bacterial growth and susceptibility testing.

Statistical analyses:

Statistical analyses were done using the SPSS 25.0 version software. Chi-square test was used to compare recovery rate of *V. cholerae* from the rectal swabs taken at the CTC of the Kanyaruchinya IDP camp with those taken from patients around Goma city. A p value < 0.05 was considered statistically significant.

Results:

Fig 1 shows a map of the North-Kivu province, with IDP camps and settlements affected by the outbreak. A total of 807 and 206 *V. cholerae* isolates were cultured respectively from the 1604 rectal swabs samples at the Kanyaruchinya IDP camp, and 538 rectal swab samples at the settlements around the city of Goma. The corresponding positivity rates were 50.3% and 38.3% respectively. The difference in culture positivity rates between Kanyaruchinya IDP camp and around the city of Goma was statistically significant (p < 0.01).

All the *V. cholerae* isolates from Kanyaruchinya IDP (n=807, 100.0%) were serotyped as *V. cholerae* O1 Inaba (i. e. agglutinated simultaneously with *V. cholerae* O1 Poly and Inaba antisera) whereas 136/206 (66%), 67/206 (32.5%), and 3/206 (1.5%) *V. cholerae* isolates from settlements around the city of Goma were serotyped as Ogawa (i. e. agglutinated simultaneously with *V. cholerae*

Table 1: Comparative antimicrobial susceptibility profiles of selected *Vibrio cholerae* O1 serotypes isolated from patients from Kanyaruchinya IDP camp and around the City of Goma

Location	**Kanyaruchinya IDP camp**		**City of Goma**					
Serotypes	**Inaba (n=174)**		**Inaba (n=20)**		**Ogawa (n=41)**		**Hikojima (n=1)**	
Antibiotics	**No sensitive (%)**	**No resistant (%)**	**No sensitive (%)**	**No resistant (%)**	**No sensitive (%)**	**No resistant (%)**	**No sensitive (%)**	**No resistant (%)**
Ampicillin	0	174 (100.0)	7 (35.0)	13 (65.0)	14 (34.1)	27 (65.9)	0	1 (100.0)
Ciprofloxacin	174 (100.0)	0	14 (70.0)	6 (30.0)	27 (65.9)	14 (34.1)	1 (100.0)	0
Norfloxacin	174 (100.0)	0	14 (70.0)	6 (30.0)	27 (65.9)	14 (34.1)	1 (100.0)	0
Azithromycin	174 (100.0)	0	20 (100.0)	0	41 (100.0)	0	1 (100.0)	0
Tetracycline	174 (100.0)	0	20 (100.0)	0	41 (100.0)	0	1 (100.0)	0
Doxycycline	174 (100.0)	0	20 (100.0)	0	41 (100.0)	0	1 (100.0)	0
Chloramphenicol	0	174 (100.0)	0	20 (100.0)	0	41 (100.0)	0	1 (100.0)
Cotrimoxazole	0	174 (100.0)	0	20 (100.0)	0	41 (100.0)	0	1 (100.0)

O1 Poly and Ogawa antisera), Inaba (i. e. agglutinated simultaneously with *V. cholerae* O1 Poly and Inaba antisera), and Hikojima (i. e. agglutinated simultaneously with *V. cholerae* O1 Poly; Ogawa, and Inaba antisera) respectively.

Antimicrobial susceptibility tests performed on a total of 236 selected *V. cholerae* isolates [174 selected randomly from the 807 isolates collected at the Kanyaruchinya IDP camp, and 62 from the settlements around the city of Goma (with 41 *V. cholerae* O1 serotype Ogawa, 20 *V. cholerae* O1 Inaba serotype, and 1 *V. cholerae* O1 serotype Hikojima) is shown in Table 1. All the tested *V. cholerae* isolates (100.0%) displayed reduced susceptibility to cotrimoxazole. All the tested from the Kanyaruchinya IDP camp (n=174) were susceptible to ciprofloxacin, norfloxacin, tetracycline, doxycycline, and azithromycin, but displayed reduced susceptibility to ampicillin, and chloramphenicol.

The antimicrobial susceptibility profiles of the 62 *V. cholerae* isolates from patients around the city of Gomawere were slightly different from the patterns of isolates from the Kanyaruchinya, with 35.0% of *V. cholerae* O1 Inaba isolates susceptible to ampicillin, 100% to tetracycline and doxycycline, and 100% resistant to chloramphenicol. With respect to ciprofloxacin, 70% and 30% of isolates were susceptible and resistant respectively. Of the *V. cholerae* O1 Ogawa isolates (n=41), 100% were resistant to chloramphenicol, while the remaining were susceptible to tetracycline, doxycycline, and azithromycin, and 34.1% and 65.9% were susceptible and resistant to ampicillin respectively, while 65.9% and 34.1% of Ogawa isolates were susceptible and resistant to ciprofloxacin respectively.

Discussion:

Since the in influx of Rwandan refugees in DRC in 1994, cholera has become a major healthcare issue in the eastern part of the country, especially in the basins of Lakes Kivu (which includes Goma and its surroundings), and Tanganyika (17). Phylogenomics analyses on *V. cholerae* O1 isolates in eastern DRC provinces in recent studies have shown that a lineage of *V. cholerae* O1 Inaba serotype corresponding to ST515 or AFR10d was the sole lineage associated with cholera outbreaks in the basin of Lake Kivu, whereas the AFR10d coexisted with a ST69 lineage corresponding to AFR10e in the basin of Lake Tanganyika (16,17). In the recent years however, the AFR10d was apparently extinct around Lake Tanganyika (16), whereas no data on isolates in the basin of Lake Kivu were available.

Our daily routine work on *V. cholerae* isolated in settlements around the city of Goma suggest a similar trend to 2021-2022, as only *V. cholerae* O1 Ogawa isolates were characterized in our laboratory (data not shown). Our data show that probably one *V. cholerae* O1 Inaba lineage was responsible of the cholera outbreak in the Kanyaruchinya IDP camp, whereas in settlements around the city of Goma, at least three lineages (*V. cholerae* O1 Inaba, *V. cholerae* O1 Ogawa, and *V. cholerae* O1 Hikojima) co-existed during the cholera outbreak. We hypothetize

that *V. cholerae* O1, Inaba serotype from patients in IDP who left their homes in the Rutshuru district more than fifty kilometers north of Goma, and who found shelter in the Kanyaruchinya IDP camp before pushing further south towards the city of Goma and its sorroundings, might have re-introduced the *V. cholerae* O1 Inaba lineage in these settlements around Lake Kivu where *V. cholerae* O1 Ogawa was already present.

The characterization of isolates of the Hikojima is somewhat startling, and to the best of our knowledge has never been reported prior to this study. The characterization of the three isolates of Hikojima phenotype warrants further investigation as this phenotype has never been characterized in the North-Kivu province. It is expected that future genomic characterization of these Hikojima serotypes will help to get insight into circulation of *V. cholerae* O1 lineages in eastern provinces of DRC and their relationships with other serotypes in the region.

In our study, only 34.1 % of Ogawa isolates were resistant to ciprofloxacin. This finding is intruiging, considering that recent data from Lake Tanganyika basin have shown that Ogawa isolates belonging to the the AFR10e (ST69) lineage displayed reduced susceptibility to ciprofloxacin (16). The difference in culture yield of *V. cholerae* between the Kanyaruchinya IDP camp samples and those from settlements around Goma is difficult to explain, considering the fact that the same teams performed the faecal sampling. Indeed between 50.3% of rectal swabs cultured positive for *V. cholerae* O1 in Kanyaruchinya IDP camp compared to 38.3% from samples collected around Goma, and which is consistent with previous studies in the region (15). One plausible explanation would be the fact that the laboratory had a team at the Kanyaruchinya IDP which process the faecal samples directly without any delay, which was not the case for samples coming from settlements around Goma.

Our study had several limitations. First, due to financial constraints, antimicrobial susceptibility tests could only be completed for 174 and 62 isolates selected randomly out of a total of 807 and 206 isolates from the Kanyaruchinya IDP camp and from the settlements around Goma city respectively, which fall short of the required sample size, as recommended in medical studies (18). The second limitation is the unavailability of minimum inhibitory concentration (MIC) values for all the tested antimicrobial agents as a reference method for confirmation of antimicrobial susceptibility profile of antibiotics.

While no inference on the general anti microbial susceptibility profiles of *V. cholerae* O1 associated with this cholera outbreak can be made due to the above limitations, our findings are worthy of being reported, and make a case for continuous monitoring of *V. cholerae* isolates associated with cholera in DRC. They should also serve as cautions for policy makers in DRC that antimicrobial treatment of *V. cholerae* O1 infection in DRC should take into account the co-existence of several antimicrobial resistance among *V. cholerae* O1 isolates. We are convinced that antimicrobial susceptibility testing in early phase of a cholera outbreak should be used as a way to establish guidelines for antimicrobial treatment of cholera rather than adopting a national antimicrobial policy not adapted to the ever changing features of *V. cholerae* associated with cholera outbreaks in DRC.

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Contributions of authors:

RKSK and PKM designed the study; RKSK wrote the first draft of the manuscript; HKM, JMB and PKM oversaw the acquisition of data; BTM, HKM, JMB and PKM analyzed and interpreted the data; and RKSK and PKM finalized the writing of the manuscript. All authors read and approved the final version of the manuscript.

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Conflicts of interest:

No conflict of interest is declared

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