Antimicrobial resistance of Vibrio cholerae O1 strains isolated during cholera epidemics in eastern Democratic Republic of Congo between January 2011 and June 2022

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Research Article

Keywords: Vibrio cholerae O1, antibiotic resistance, resistance profile, eastern DRCongo

Posted Date: January 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2456709/v1

Abstract

Background

Although the mainstay of cholera treatment remains rehydration, treatment with antibiotics is recommended in serious forms and on specific sites. However, since the late 1970s, resistance to antibiotics in strains of *Vibrio cholerae* (Vc) has been observed and now threatens the effective treatment and control of cholera.

Objective and Methods

This cross-sectional, retro-prospective, descriptive and analytical study aims to describe the long-term evolution of the antibiotic resistance profile of *Vibrio cholerae* O1 strains isolated at the provincial public health laboratory of North Kivu during cholera epidemics in Eastern Democratic Republic of Congo (DRC) from January 1, 2011 to June 30, 2022.

Results

A total of 4832 isolates of *Vibrio cholerae* O1 were collected, more than half of them (72.46%, n=3175) in the province of North Kivu. Of the 3 serotypes, Vc O1 Inaba was the majority (57.6%, n=2522). Among the 4382 strains of Vc O1, very high levels of resistance were found to ampicillin (74.7%), nalidixic acid (83.8%), erythromycin (73.6%), chloramphenicol (68.8%) and Sulfamethoxazole/Trimethoprim (82%). A resistance of 30% (1316/4382) was found to doxycycline, a molecule recommended by the WHO as first-line in the treatment of cholera. We found 27 different resistance profiles (MDR) with respect to the 5 main molecules recommended in anticholera therapy. In the analytical study, we observed a statistically significant evolution over time (p=0.000) of these MDR profiles, including 5% in 2011 against nearly 40% between 2021 and 2022; with a distribution statistically dependent on age (p=0.0003) including 57.4% of cases in children under 15 (27% in those under 5); with a predominance (69%) in the province of North Kivu.

Conclusion

The resistance of *Vibrio cholerae* 01 to common antibiotics is high in eastern DRC and tends to increase over time. The abusive and inappropriate use of antibiotics is one of the major causes of this emergence of antimicrobial resistance. Close monitoring and measures for the proper use of antibiotics will be necessary to stem this scourge.

Background

Cholera is a highly contagious acute intestinal poisoning caused by a gram-negative bacterium called *Vibrio cholerae* [1]. This possesses multiple virulence factors, including cholera toxin and the pilus co-regulated with the toxin, responsible for the typical symptoms of the disease [2]. Nearly 4 million cases of cholera are reported each year worldwide, including 189,000 in the Democratic Republic of Congo (DRC).

The eastern provinces, known as “hot spots”, reporting more than half of these cases. Although the mainstay of cholera treatment remains rehydration, antibiotic therapy is recommended in more severe forms and sometimes in patients with associated medical conditions (including pregnancy) or suffering from comorbidities (eg, severe acute malnutrition, HIV) who present a high risk in severe cases of cholera [3]. By their direct action on the vibrio, antibiotics can significantly reduce the bacterial load, thus reducing the severity and duration of symptoms [4]. However, since the late 1970s, increasing resistance to antibiotics in strains of *Vibrio cholerae* (Vc) has been observed and now threatens the optimal management of moderate to severe cases of cholera. Epidemics caused by antibiotic-resistant *Vibrio cholerae* are fraught with higher mortality [5, 6]. This is why at the start of an epidemic, an antibiogram must be carried out.

Estimated at more than 700,000 deaths per year worldwide, antimicrobial resistance (AMR) leads to treatment failure, leading to longer hospital stays, increased medical expenses and increased mortality [6]. According to the WHO, if no appropriate measures are taken to stop its progression, AMR will cost around 10 million lives and around 100 billion US dollars per year by 2050 [7, 8]. Despite the threat presented by this antibiotic resistance, the WHO and the recent O'Neill report describe major shortcomings in surveillance, standard methodologies and data sharing [8, 9]. This lack of quality data is problematic, often leading to treatment guidelines that are not adapted to the local situation [6, 10]. It is therefore important to monitor the evolution of antibiotic sensitivity of microbial strains in general and of Vc O1 in particular in each African country in order to better adapt treatment [8, 10].

In the Democratic Republic of Congo, several longitudinal studies have confirmed the permanent and growing epidemiological nature of cholera, which has become a clinical landscape in the DRC [11, 12–14]. However, few of them have been interested in evaluating changes in the resistance to antibiotics of *Vibrio cholerae* O1 in this region. It is in this context that our present study takes place, the main objective of which is to describe the long-term evolution of the antibiotic resistance profile of the strains of *Vibrio cholerae* O1 isolated during cholera epidemics in the East of the DRC.

Materials And Methods

We conducted a cross-sectional, retro-prospective, descriptive and analytical study on the strains of *Vibrio cholerae* O1 isolated during the cholera epidemics that affected eastern DRC from January 1, 2011 to June 30, 2022. Were included in this study, all isolates positive for *Vibrio cholerae* O1 from the eastern provinces of the DRC and registered at the provincial public health laboratory of North Kivu AMI LABO during our study period.

The strains of *Vibrio cholerae* were cultured on TCBS nutrient agar, enriched in a liquid medium with alkaline peptone water and incubated at 37° C. for 18 to 24 hours. Morphological, biochemical and serogroup characterizations were then performed according to conventional bacteriological techniques and standard protocols [15, 16, 17]. *Vibrio cholerae* O1 isolates were tested against ten antibiotics: Ampicillin (25 µg), Tetracycline (30 µg), Doxycycline (30 µg), Erythromycin (15 µg), Azithromycin (15 µg),

Nalidixic acid (30 µg), Ciprofloxacin (5 µg), Norfloxacin (5 µg), Chloramphenicol (30 µg), Sulfamethoxazole/Trimethoprim (1.25/23.75 µg). These tests were performed using the Kirby-Bauer disk diffusion method with Mueller Hinton agar. The interpretation of the inhibition diameters was made according to the guidelines of the Clinical and Laboratory Standards Institute CLSI (Sensitive, Intermediate and Resistant). Quality control was performed using Escherichia coli ATCC 25922 [18, 19]. The intermediate profile was interpreted and analyzed as is. Taking into account the 5 molecules used in first intention in the treatment of cholera according to the WHO recommendations, we grouped the resistance profiles according to these 5 antibiotics: Doxycycline, Azithromycin, Erythromycin, Ciprofloxacin and Tetracycline.

Data was collected from the "cholera" registers of the bacteriology department of the provincial public health laboratory of North Kivu (AMI LABO) using a standard collection sheet including socio-demographic characteristics (age, sex, geographical origin, period) and biological (type of sample, examination in the fresh state including mobility, gram staining, culture, serogrouping, serotyping, biotyping), as well as the result of the antibiogram.

Data were entered into Microsoft Excel software and analyzed using R version 4.0.3 software. Spatio-temporal analysis was performed based on the resistance profiles using Seaborn, Matplotlib and Numpy packages of Python version 3.8 software. Categorical variables were described as relative and absolute frequencies. As for the quantitative variables, they were presented in the form of mean ± standard deviation or median depending on the type of distribution. A bivariate analysis was determined using Fisher-Freeman-Halton's exact test and Pearson's chi-square. Values of $p < 0.05$ were considered statistically significant.

Results

Four thousand three hundred eighty two (4382) isolates of *Vibrio cholerae* O1 were included in this study. All 3 serovars were found: Vc O1 Inaba (57.6%, n = 2522), Vc O1 Ogawa (40.6%, n = 1777) and Vc O1 Hikojima (1.9%, n = 83), as shown in Fig. 1 below.

However, Table I below summarizes the origin of these 4382 isolates recorded at the provincial public health laboratory of North Kivu AMI LABO, as well as their distribution according to the age and sex of the patients.

Table I. *Vibrio cholerae* O1 strains isolated according to age, sex and provenance (n = 4382).

Variables	*Vibrio cholerae* O1 strains isolated (n=4382)			Total	p-value
	Vc O1 Hikojima	Vc O1 Inaba	Vc O1 Ogawa		
	n (%)	n (%)	n (%)	n (%)	
Age (year)					
< 5	25(2,1)	594(50,0)	569(47,9)	1188(100)	
5 – < 15	26(1,9)	740(54,9)	581(43,1)	1347(100)	
15 - < 25	7(1,0)	439(62,9)	252(36,1)	698(100)	**< 0,001**
≥ 25	25(2,2)	749(65,2)	375(32,6)	1149(100)	
Sex					
Male	47(2,1)	1259(55,8)	952(42,2)	2258(100)	**= 0,040**
Female	36(1,7)	1263(59,5)	825(38,8)	2124(100)	
Provinces					
Kasaï Oriental	0(0,0)	0(0,0)	11(100)	11(100)	
Lomami	0(0,0)	0(0,0)	1(100)	1(100)	
Maniema	0(0,0)	2(100)	0(0,0)	2(100)	**< 0,001**
Nord-Kivu	30(0,9)	2134(67,2)	1011(31,8)	3175(100)	
Sud-Kivu	53(4,6)	348(30,3)	747(65,1)	1148(100)	
Tanganyika	0(0,0)	38(84,4)	7(15,6)	45(100)	

Indeed, it appears from this table that half (51.5%) of the patients were male. The mean age was 17.25 ± 17 years (0–90 years). The age group of 5 to 14 years was the most affected (30.7%) followed by that of 0 to 4 years (27.1%). Children aged 0 to 14 alone accounted for more than half of the cases with a total of 57.8%. More than half (72.46%) of the patients came from the province of North Kivu. The distribution of these 3 strains of *Vibrio cholerae* O1 isolated during this study was statistically dependent on the age, sex and province of origin of the patients, but also related to an evolution over time (p = 0.000) (Fig. 2).

Susceptibility test of isolated Vibrio cholerae **O1 strains**

The *Vibrio cholerae* O1 strains isolated in this study showed fairly high levels of resistance to several antibiotics, namely ampicillin (74.7%), nalidixic acid (83.8%), chloramphenicol (68.8%) and Sulfamethoxazole/Trimethoprim (82%). Cyclins, quinolones and macrolides remained relatively sensitive,

but with worrying levels of resistance because they are first-line molecules in the management of cholera. For this purpose, doxycycline, a first-line molecule as recommended by the WHO and the GTFCC, was 30% resistant (Table II).

Table II. Distribution of isolated *Vibrio cholerae* O1 strains against ten antibiotics tested (n = 4382).

Antibiotics	*Profil*				
	ND	**I**	**S**	**R**	**Total**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**N (%)**
TE	539 (12,3)	92 (2,1)	3117 (71,1)	634 (14,5)	4382 (100)
AM	556 (12,7)	254 (5,8)	297 (6,8)	3275 (74,7)	4382 (100)
CI	198 (4,5)	239 (5,5)	3612 (82,4)	333 (7,6)	4382 (100)
SXT	393 (9,0)	34 (0,8)	361 (8,2)	3594 (82,0)	4382 (100)
C	435 (9,9)	120 (2,7)	813 (18,6)	3014 (68,8)	4382 (100)
NA	358 (8,2)	118 (2,7)	236 (5,4)	3670 (83,8)	4382 (100)
E	450 (10,3)	384 (8,8)	323 (7,4)	3225 (73,6)	4382 (100)
DO	718 (16,4)	146 (3,3)	2202 (50,3)	1316 (30,0)	4382 (100)
NOR	273 (6,2)	115 (2,6)	3825 (87,3)	169 (3,9)	4382 (100)
AZ	2336 (53,3)	31 (0,7)	1949 (44,5)	66 (1,5)	4382 (100)

TE: Tetracycline, **AM**: Ampicillin, **CI**: Ciprofloxacin, **SXT**: Sulfamethoxazole/Trimethoprim, **C**: Chloramphenicol, **NA**: Nalidixic Acid, **E**: Erythromycin, **DO**: Doxycycline, **NOR**: Norfloxacin, **AZ**: Azithromycin. **I**=intermediate, **ND**=Not determined, **R**=resistant, **S**=susceptible.
In addition, Fig. 3 below summarizes the distribution of the 3 different serotypes of *Vibrio cholerae* O1 which were isolated in this study according to the results of the antibiogram against each of the 10 antibiotics tested.

However, taking into account the 5 molecules used in first intention in the treatment of cholera according to the WHO recommendations, we grouped the resistance profiles according to these 5 antibiotics: Doxycycline, Azithromycin, Erythromycin, Ciprofloxacin and Tetracycline. Thus, we observed 27 different profiles with respect to these 5 molecules. As shown in Figs. 4 and 5, the Inaba strain had statistically higher multiple antibiotic resistance (MDR) levels ($p = 0.000$) compared to the other 2 (Ogawa and Hikojima). These different resistance profiles were statistically dependent on age ($p = 0.0003$) with 57.4% of MDR cases in children under 15 years old (including 27% in those under 5 years old).

A spatio-temporal analysis was carried out according to these different resistance profiles (Fig. 6).

The circumference or size of the bubbles represents the relative number of strains, the columns and rows correspond to the period and the different resistance profiles respectively, while the colors correspond to the provinces.

This figure shows that the majority of resistance profiles came from the provinces of North and South Kivu; with a relative increase in MDR profiles over time (p = 0.000).

Discussion

Epidemics with resistant strains are burdened with higher mortality [20]. Several studies have reported quasi-concordant data on the emergence of strains of *Vibrio cholerae* O1 resistant to antibiotics. In a recent Indian meta-analysis, Chatterjee P et al [21], reported 62 outbreaks of cholera epidemics with resistant strains in India between 2009 and 2012 with an increasing trend over time of antibiotic resistance of *Vibrio cholerae*. In Ghana, Kuma KG et al [22] reported very high resistance rates of *Vibrio cholerae* O1 to cotrimoxazole (96.3%) and erythromycin (94.4%), then low to Azithromycin (0%), ciprofloxacin (0.4%), doxycycline (14.5%) and tetracycline (15.6%). Similarly, Ingebelbeen B et al [23] observed 99.6% resistance to cotrimoxazole and 67.4% to erythromycin. At the same time, Mandomando I et al [24] reported 96.6% resistance to cotrimoxazole, 97.3% to tetracycline and 58% to chloramphenicol; like Rijal N et al [25], who observed 100% of *Vibrio cholerae* O1 strains resistant to co-trimoxazole and nalidixic acid. For their part, Ndoutamia G et al [26] reported that among the resistance profiles of the etiological agents of diarrhea isolated in Chad, *Vibrio cholerae* O1 showed resistance to tetracycline (4.01%), doxycycline (4.37%) and ciprofloxacin (8.52%). Bactrim, chloramphenicol, and nalidixic acid were virtually inactive.

Our results are perfectly complementary to these observations. For penicillins, 74.7% (3275 of 4382 cases) of *Vibrio cholerae* O1 strains isolated in our study were resistant to ampicillin. Quinolones/fluoroquinolones remained predominantly susceptible with 87.3% (3825 out of 4382 cases) and 82.4% (3612 out of 4382 cases) strains susceptible to norfloxacin and ciprofloxacin respectively; but with 83.8% resistance (3670 out of 4382 cases) to nalidixic acid. Resistance to cyclins was 30% (1316 out of 4382) for the first-line molecule doxycycline in the treatment of cholera and 14.5% (634 out of 4382) for tetracycline. Regarding macrolides, the majority (3225 out of 4382, ie 73.6%) of the strains were resistant to erythromycin and 1.5% to azithromycin with a sensitivity of 44.5%. Chloramphenicol and sulfamethoxazole/trimethoprim were predominantly resistant at 68.8% and 82% respectively.

Our results show a lower sensitivity rate to cyclines and fluoroquinolones compared to the series of Ingelbeen B et al [23], who reported sensitivity rates of 99.2% to doxycycline, 99.1% to tetracycline and 96.9% to ciprofloxacin. The realization of the antibiogram interesting azithromycin had started from the year 2018 of our study by the AMI LABO laboratory, which would explain more than 50% of the undetermined results. In addition, we analyzed the intermediate profile as is, unlike several studies that classified this profile as associated with resistance.

Moreover, in several other studies, ciprofloxacin, doxycycline, and azithromycin remained the most sensitive molecules. This corresponds to data from the WHO and the GTFCC which recently recommended the use of the five molecules in the management of cholera, namely fluoroquinolones (ciprofloxacin), cyclins (doxycycline, tetracycline) and macrolides (azithromycin, erythromycin) [27].

Taking this WHO recommendation into account in our study, according to the antibiogram data, we found 27 different resistance profiles representing 3515 strains of *Vibrio cholerae* O1. Of these, 8 (i.e. 0.23%) were resistant to the 5 molecules at the same time (Profile_17), 7 (87.5%) of them were of the Inaba serotype and all were isolated in 2021–2022. Among these strains, 141 (4%) were resistant to ciprofloxacin, erythromycin and doxycycline at the same time and 268 (ie 7.6%) to three molecules at the same time, including the doxycycline recommended as first-line treatment. Variable resistance to several first-, second-, and third-line antibiotics has been found, making *Vibrio cholerae* O1 one of the currently multi-resistant (MDR) bacteria. Overall, our study observed an evolution of these profiles over time with a statistically significant difference (p = 0.000), including 165 multiresistant strains in 2011 (i.e. nearly 5%) against 1403 MDR strains (i.e. nearly 40%) between 2021 and 2022. The profile_14 being resistant both to the two cyclins tested and to erythromycin, increased from 9.7% in 2011 to 14.2% between 2021 and 2022.

In relation to age, we observed a statistically significant difference (p = 0.0003) in the distribution of these MDR profiles with 57.4% of cases in children under 15 years of age (including 27% in those of less than 5 years). However, we did not find any statistically significant difference between these profiles and gender. On the other hand, the distribution of these 27 resistance profiles was statistically dependent on the provinces, with a predominance in the province of North Kivu (2426 out of 3515 cases, or 69%). In this regard, our results can be superimposed on those of Miwanda B et al [28], who reported 21 resistance profiles of *Vibrio cholerae* O1 strains isolated in the DRC between 1997 and 2012 while considering the intermediate profile as being resistant with a significant growth over time. For their part, Igere BE et al [29], reported MDR profiles of so-called "critical" priority by the WHO, including strains of resistant *Vibrio cholerae* producing metallo-betalactamase type NDM carbapenemases, extended-spectrum beta-lactamases and various other resistant genotypes/phenotypes.

Nalidixic acid is most often used to detect low-level resistance to fluoroquinolones, which can become high-level if there is an additional effect of chromosomal mutation and plasmid protection. Thus, the very high prevalence of resistance to nalidixic acid found in our series, as in others, could influence sensitivity to ciprofloxacin and norfloxacine.

Conclusion

It appears from this study that cholera remains a real major public health problem in eastern DRC and the resistance to antibiotics of strains of Vibrio cholerae O1 would further complicate its management. Variable resistance to first, second and third-line antibiotics has been found and tends to increase over time, thus making *Vibrio cholerae* O1 one of the currently emerging multidrug-resistant (MDR) bacteria in

this part of the DRC. A resistance of 30% (1316/4382) was found to doxycycline, a molecule recommended by the WHO as first-line in the treatment of cholera. The province of North Kivu being the most affected. Resistant *Vibrio cholerae* O1 predominated more in children under 5 years old. Epidemics caused by antibiotic-resistant *Vibrio cholerae* have been shown in several studies to be associated with higher mortality [14, 16]. Given that half of cholera cases and deaths occur in children under 5 years of age [5, 8], the increase in antibiotic resistance in this child population would further complicate anticholera therapy. Inappropriate antibiotic prescriptions for patients with diarrhea, availability of over-the-counter antibiotics without a valid prescription, and consumption of inappropriate or incomplete antibiotic regimens could contribute to this emergence of resistant *Vibrio cholerae*. In this context, close monitoring and measures for the proper use of antibiotics will be necessary to stem this scourge. The development of effective oral cholera vaccines (OCV) also presents an interesting policy alternative as demonstrated by several studies [53,55]. Multisectoral surveillance of antibiotic resistance in clinical and environmental strains of *Vibrio cholerae* is also important, thus integrating the "One Health" approach, a major pillar in the fight against antibiotic resistance [19].

Future Prospects

Considering that the effect of the targeted use of antibiotics for cholera on the resistance profile of potentially pathogenic enteric bacteria also present at the time of treatment remains unknown, it seems reasonable to us:

- To carry out an analysis of the strains of *Vibrio cholerae* O1 of clinical and environmental origin, by molecular biology tools (sequencing, genotyping, phylogenetic analysis) thus allowing on the one hand to acquire information on the resistance phenotypes and on the other hand, to distinguish the phenomena of clonal diffusion of resistant strains or the transfer of resistance genes between *Vibrio cholerae* and other pathogenic enterobacteriaceae.

Limitations Of The Study

This study presented some limitations: first, we noted the absence of clinical data from patients since the study was carried out in the laboratory; then the absence of data on the methods of contamination of our patients, which could allow a good targeted strengthening of the management of cholera cases. Also, the lack of certain antibiogram data with several undetermined or unrecorded values.

Declarations

Acknowledgements

The authors are grateful to all the staff of the public health laboratory of North Kivu AMI LABO for having actively participated in this study and allowing it to be carried out in complete peace of mind.

Author contributions

PAN conceived of the study, and participated in its data collection, statistical analysis, design and drafting. BWS, FW, AB, LD performed the statistical analysis. JBM, HMK, participated in the laboratory examination and data collection. RKKS supervised all phases of laboratory examination and data collection. VMPC, RCKT helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study obtained the authorization of the steering committee in charge of training and research within the provincial public health laboratory of North Kivu AMI LABO where the study was carried out.

Competing interests

The authors declare that they have no competing interests.

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Figures

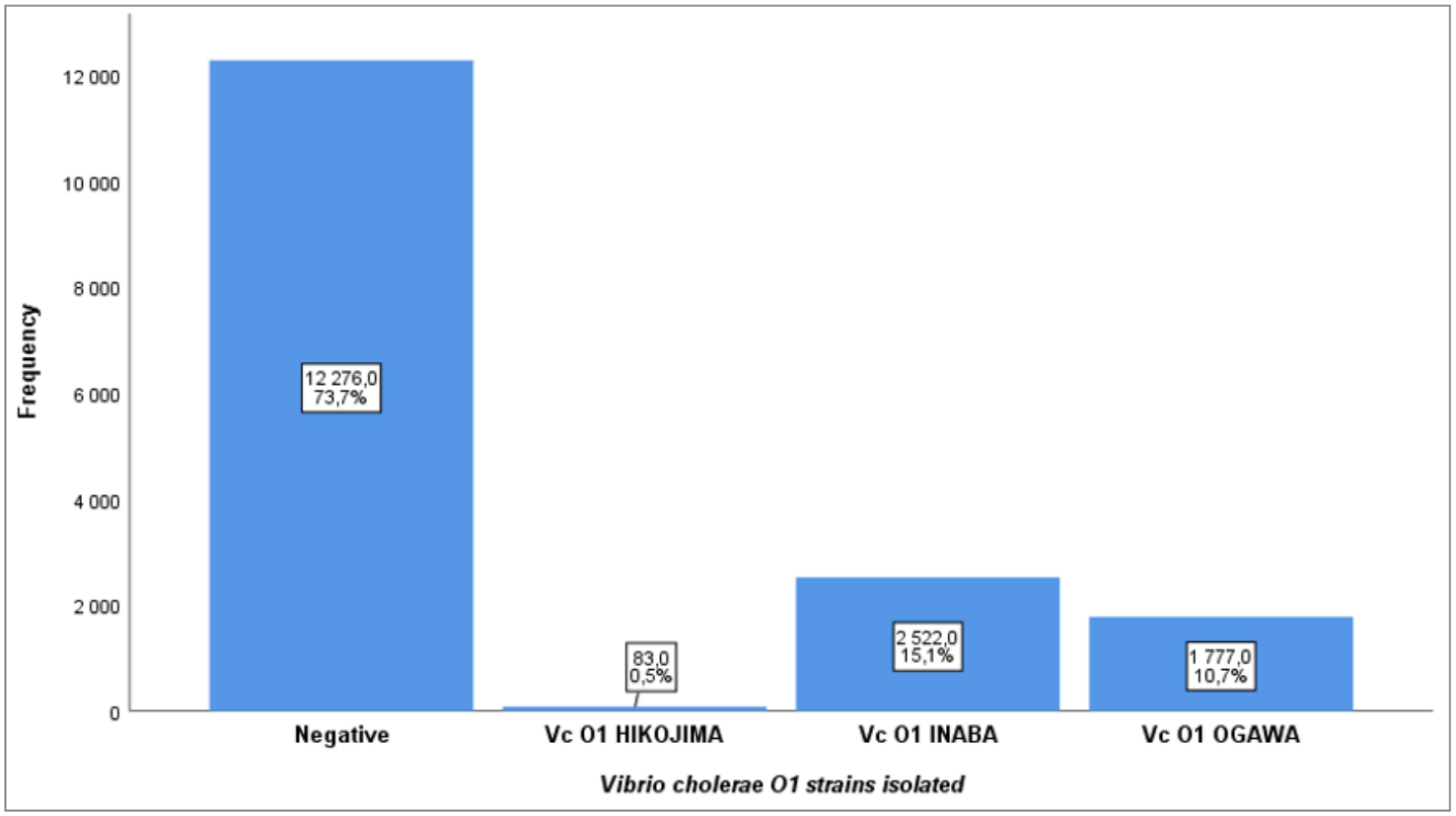

Figure 1

Distribution of *Vibrio cholerae* 01 strains isolated (n=4382).

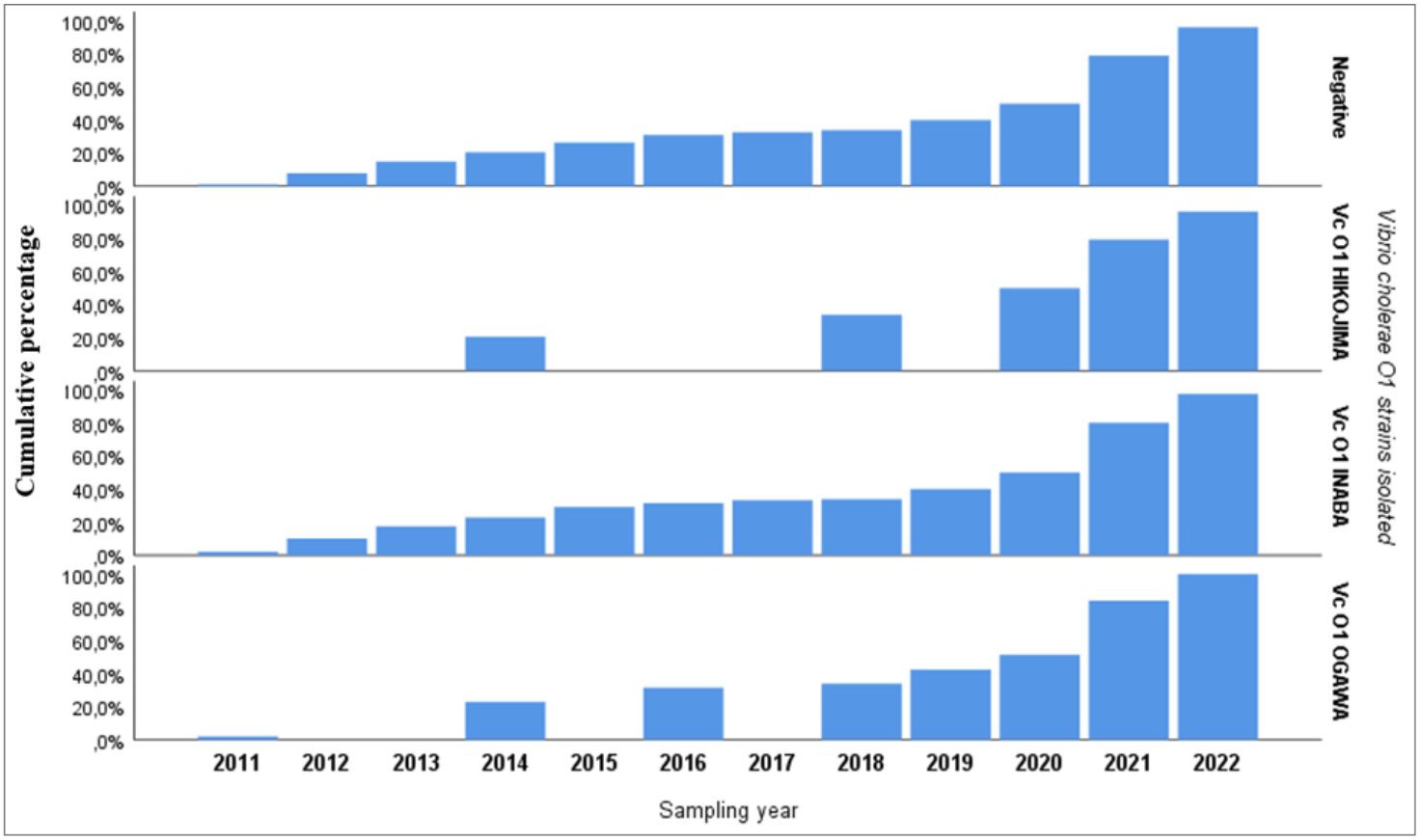

Figure 2

Distribution by period of *Vibrio cholerae* 01 strains isolated (n=4382).

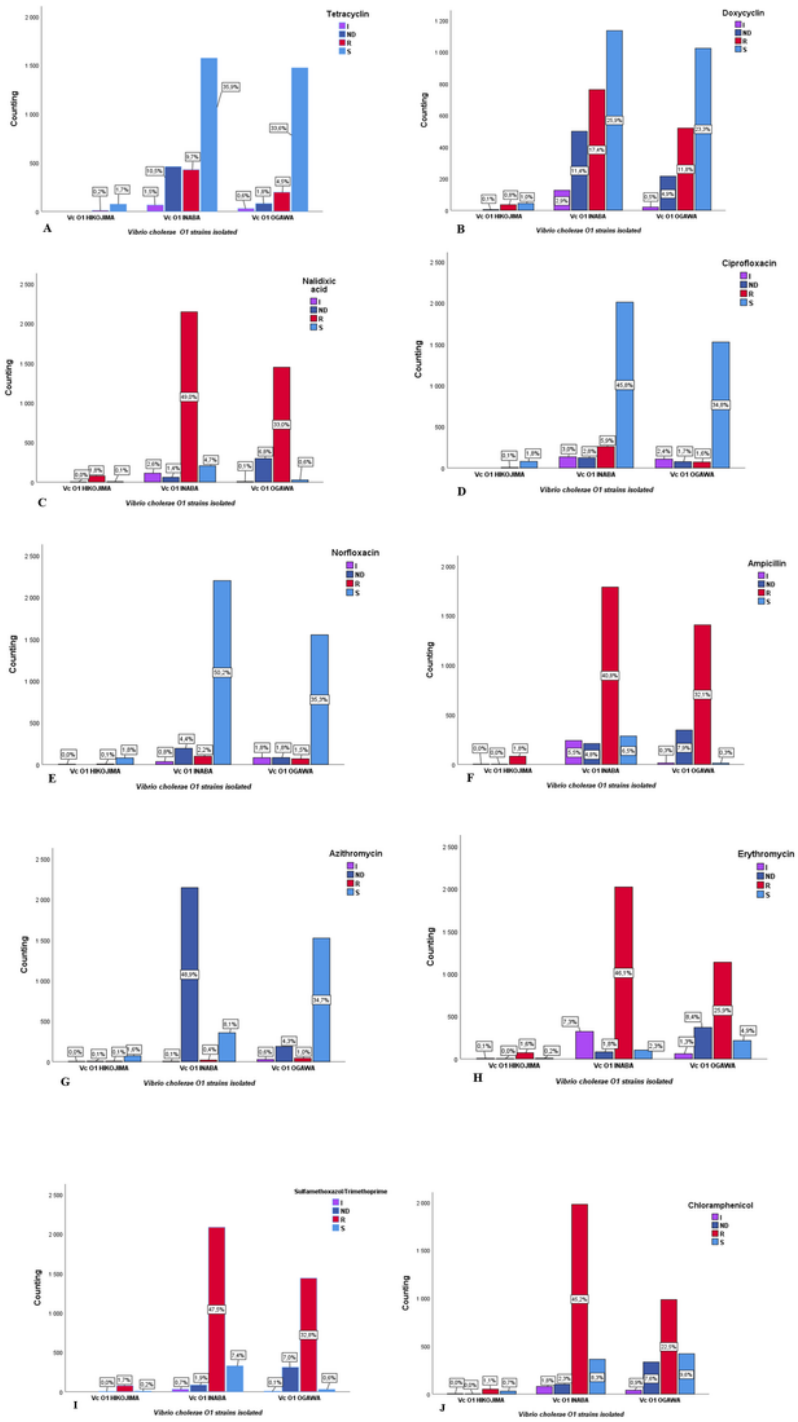

Figure 3

A-J. Antibiogram results for each antibiotic tested according to the 3 serotypes of *Vibrio cholerae* O1 isolated.

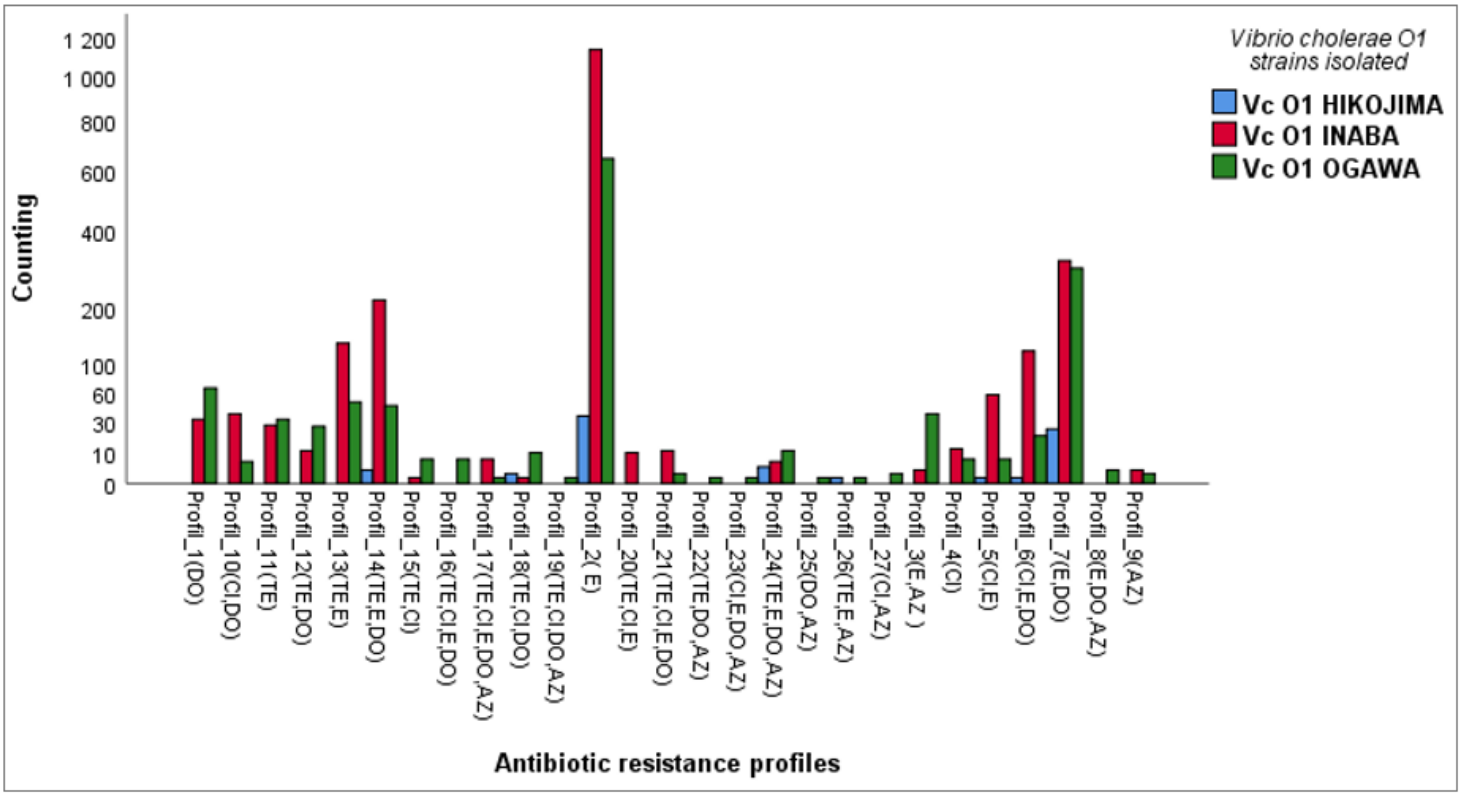

Figure 4

Distribution of the different resistance profiles grouped in relation to the 5 molecules currently recommended by the WHO for the treatment of cholera according to the serotypes of *Vibrio cholerae* O1 (n=27 profiles).

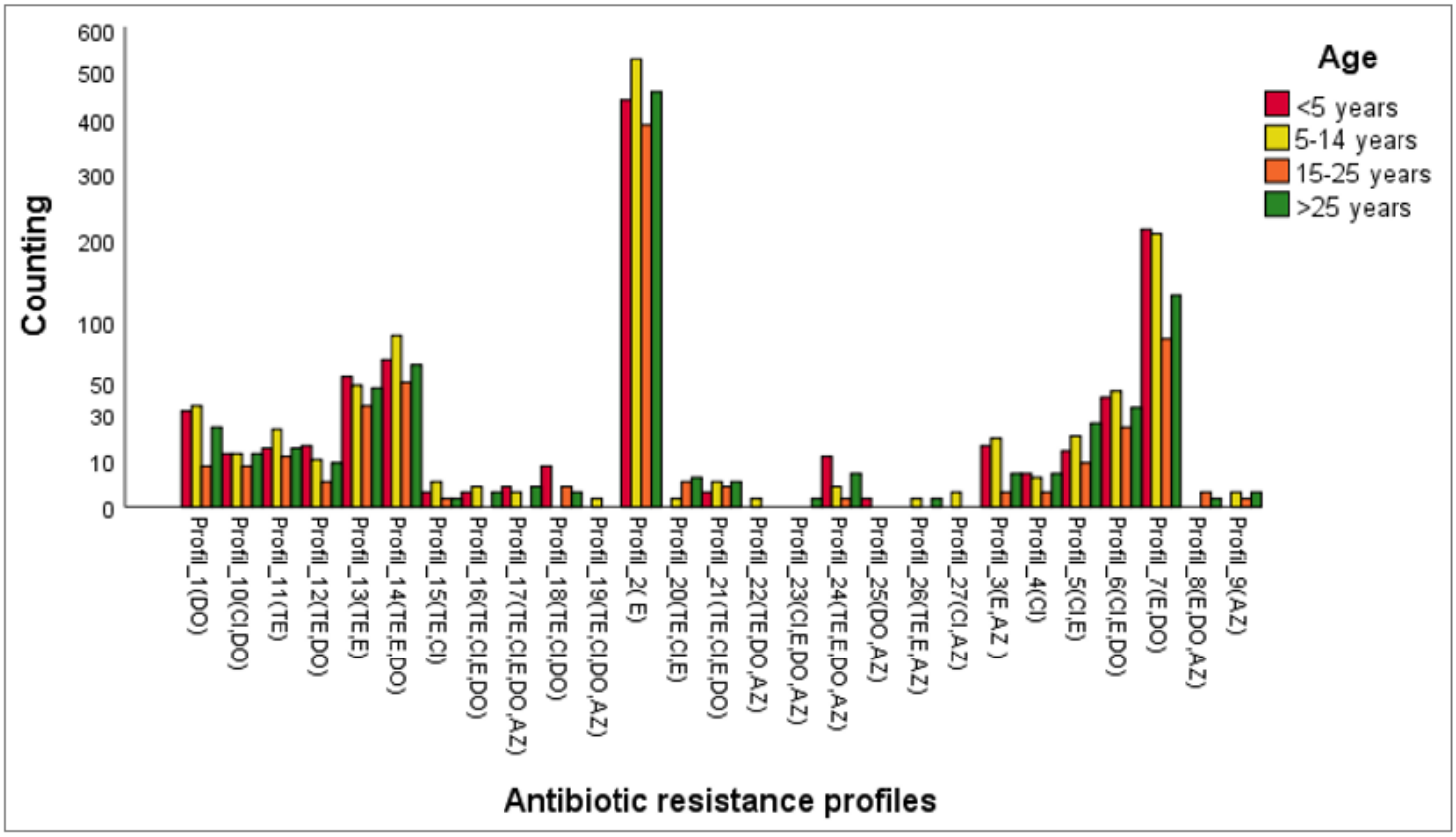

Figure 5

Distribution by age of the different *Vibrio cholerae* O1 resistance profiles (n= 27 profiles).

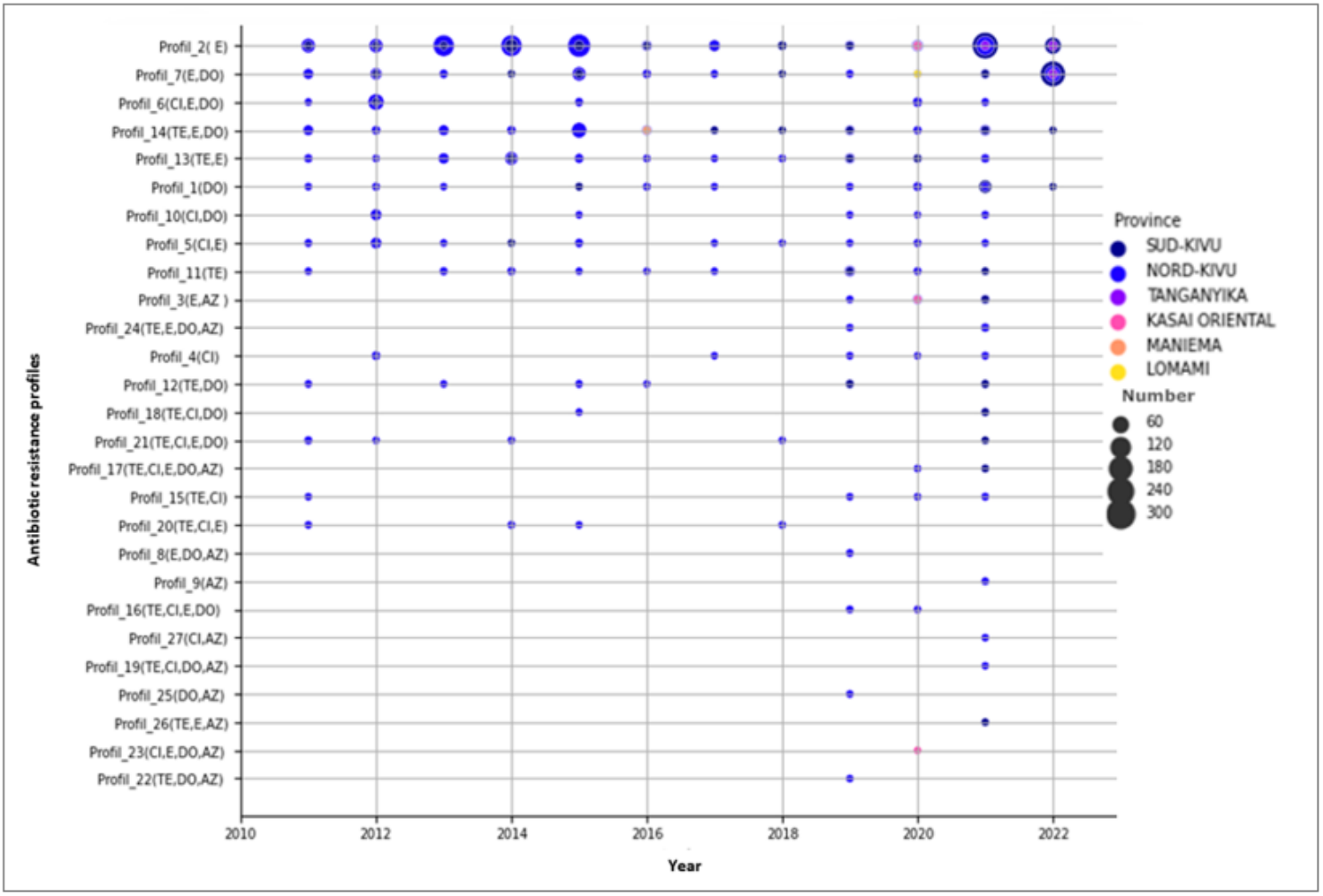

Figure 6

Spatio-temporal distribution of the different resistance profiles of *Vibrio cholerae* O1 isolates (n =27 profiles).