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EVOLVING ANTIMICROBIAL RESISTANCE IN VIBRIO CHOLERAE: INSIGHTS FROM KENYA, 2015–2023

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EVOLVING ANTIMICROBIAL RESISTANCE IN *VIBRIO CHOLERAE*: INSIGHTS FROM KENYA, 2015–2023

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ABSTRACT

Background: **Antimicrobial resistance (AMR) is a significant public health problem, with a considerable number of attributable and associated deaths both globally and in Kenya. There is an inherent data gap on AMR for *Vibrio cholerae* in Kenya. The present study aimed to document the pattern of Antimicrobial Resistance to *Vibrio cholerae* in Kenya.**

Methods: **This study used a retrospective, descriptive, and cross-sectional design. Data on the antimicrobial susceptibility and resistance of *V. cholerae* to various antibiotics were extracted from the National Public Health Laboratory (NPHL) of the Ministry of Health, Kenya, between 2015 and 2023. The data were subjected to descriptive analysis to determine the rates of sensitivity, intermediate resistance and antimicrobial resistance.**

Results: **The findings reveal that *V. cholerae* isolates were highly sensitive to chloramphenicol (94.0%), tetracycline (90.2%), gentamicin (86.8%), levofloxacin (94.1%), and cefuroxime (85.5%). Further, the isolates were highly resistant to Ampicillin (94.0%), Nalidixic acid (100%), Erythromycin (77.3%), and Ceftazidime (66.7%). Over two thirds (68.3%) of the isolates exhibited AMR, while 31.7% showed resistance to only one type of antibiotic. Among the isolates that exhibited resistance, 40.7% were resistant to two antibiotics, while 27.6% were resistant to more than two antibiotics. The predominant AMR pattern overall was Ciprofloxacin + Cefepime, while Ampicillin + Ciprofloxacin + Nalidixic acid was the predominant pattern of resistance to more than two antibiotics.**

Conclusion: **This study demonstrates an increasing trend of Antimicrobial Resistance (AMR) among *Vibrio cholerae* isolates in selected Kenyan counties, particularly to commonly used antibiotics, such as ampicillin, nalidixic acid,**

and erythromycin. Conversely, high sensitivity was observed for chloramphenicol, levofloxacin, and tetracycline. Notably, a significant proportion of the isolates exhibited Multi Drug Resistance (MDR), posing a serious threat to effective cholera treatment. In order to mitigate this trend, routine surveillance of AMR in *V.cholerae* is recommended along with stringent antimicrobial stewardship.
Keywords: **Antimicrobial Resistance, Sensitivity and *V. cholera*e**

INTRODUCTION

Antimicrobial resistance (AMR) is a significant public health problem worldwide. Over a Million deaths are associated with AMR, with 1.27 million deaths directly attributed to AMR. Evidence indicates that the misuse of antimicrobials in humans, animals, and plants is the main cause of AMR in bacterial strains [1]. In Africa, approximately 1.05 million deaths in 2019 were associated with AMR, with lower respiratory and thoracic infections accounting for the highest percentage, followed by bloodstream and abdominal infections [2]. According to a review by the UK government, AMR is projected to kill 10 million people annually by 2050 [3,4].

Evidence indicates that bacteria cause AMR through biochemical mechanisms, such as destruction or enzymatic modification of antibiotics, modification of antibiotic target sites, decrease in antibiotic penetration, and elimination of antibiotics from the cell by efflux pumps [5]. Based on this evidence, governments and agencies in the health sector and researchers agree that AMR is a significant problem that requires urgent, globally coordinated action plans to deal with [6,7]. Resistance to commonly prescribed antimicrobials is a major threat to the fight against cholera and other endemic bacterial diseases in Africa and worldwide[8]. Microorganisms that have been documented to exhibit resistance include *Escherichia coli, Klebsiella pneumonia, Neisseria spp, Salmonella serovars, and Vibrio cholerae* [9].

Vibrio cholerae O1 and O139 serogroups that exhibit AMR are associated with the majority of global cholera outbreaks[10,11]. In low-middle-income countries such as Kenya, there has been growing concern about the emergence of AMR *V. cholerae* strains[12]. Evidence suggests that AMR *V. cholerae* is resistant to the majority of antibiotics used in treatment, which is exhibited by considerably high treatment failures of the disease[13]. Multi Drug Resistance (MDR) enteric pathogens were first discovered in the 1960s [14,15]. In the past few decades, AMR in *V. cholerae* has mutated rapidly and spread globally, owing to the overuse and misuse of antibiotics in different sectors. Extensive drug resistance (EDR) and MDR *V. cholerae* genes are highly enriched with mobile genetic elements (MGEs) and can horizontally transfer resistance genes to other pathogenic microorganisms [12].

According to World Health Organization (WHO), there are limited publications and reports on AMR in Africa, including Kenya

(9). Evidence further suggests the need for the development of comprehensive and expounded data on AMR in *V. cholerae* [16]. In Kenya, AMR remains a significant health problem. For instance, in 2019, 8500 deaths were directly attributable to AMR, and another 37,300 deaths were associated with AMR[17]. During the recent cholera epidemics in Kenya, new *V. cholerae* strains exhibiting new resistance have emerged. Cholera is endemic to Kenya, and in the past few decades, the country has experienced three major protracted epidemics. However, there are considerable limitations to the data and information on the AMR patterns of *V. cholerae* at the national level [18]. This study aimed to document the AMR of *V. cholera* isolates in Kenya.

Methodology

Study design

This study adopted a retrospective descriptive design, assessing the susceptibility and resistance of *V. cholerae* clinical isolates.

Study area

A total of 279 isolates from 14 counties in Kenya between 2015 and 2023 were considered in the present study. The counties were selected based on the burden of cholera outbreak. The 14 counties exhibited high burden of cholera outbreak during the study period. The counties were Nairobi, Lamu, Machakos, Mandera, the Tana River, Kajiado, Murang'a, Kiambu, Kitui, Garissa, Turkana, Isiolo, and Narok.

Study period

The considered study period was between 2015 and 2023.

Cholera data source

Data on susceptibility and resistance to *V. cholerae* on various antibiotics were extracted from the National Public Health Laboratory (NPHL) of the Ministry of Health, Kenya. The AMR data for *V. cholerae* was consolidated between 2015 and 2023.

Data analysis

Descriptive analysis was performed using SPSS version 25, and bar graphs were drawn using RStudio 4.3.3. The data are presented in the tables and graphs as provided in the results section.

Ethical consideration

To ensure data privacy and protection, no personal identifiers including names, addresses and contact information or any other directly identifying information were collected or extracted from the secondary data sources. Ethical approval and authorization to conduct this study were obtained from the Mount Kenya University Ethical Review Committee (Ref: MKU/ISERC/4306) and the National Commission for Science, Technology, and Innovation (NACOSTI) (License no: NACOSTI/P/24/39710). Approval to access Cholera AMR data was obtained from the Kenya Ministry of Health Division of National Public Health Laboratory (NPHL).

Results

Socio-demographic characteristics

This study included 279 cases from 14 counties in Kenya, covering the period

between 2015 and 2023 (Table 1). The majority (44.8%) of the samples were from Nairobi County, followed by Lamu County, and the least were from Isiolo and Narok Counties. The majority (65.9%) of the samples were from male patients, whereas the least (30.5%) were from female patients. The mean age of the cholera patients was 33.73 ± 15.94 years.

Table 1: Socio-demographic characteristics of the cholera cases (n=279)

Variable	Frequency	Percentage (%)
Gender		
Male	184	65.9
Female	95	34.1
Age (years)		
33.73 ± 15.94		
County		
Nairobi	158	56.6
Lamu	48	17.2
Machakos	18	6.5
Manderra	13	4.7
Tana River	13	4.7
Kajiado	10	3.6
Murang'a	7	2.5
Kiambu	3	1.1
Kitui	3	1.1
Garissa	2	0.7
Turkana	2	0.7
Isiolo	1	0.4
Narok	1	0.4

Antimicrobial susceptibility of *V. cholerae*

More than three-quarters of the isolates were sensitive to chloramphenicol (94.0%), gentamicin (86.8%), cefuroxime (85.5%), tetracycline (90.2%), and levofloxacin (94.1%) (Table 2). Majority of the isolates were sensitive to ciprofloxacin (57.9%), amikacin (53.5%), and cefotaxime (56.0%). A large number of the isolates were resistant to ampicillin (94.0%), all the isolates were resistant to nalidixic acid (100%), erythromycin (77.3%), and ceftazidime (66.7%). Additionally, over half (61.8%, 57.7%, 57.7%, and 51.1%) of the isolates were resistant to trimethoprim–sulfamethoxazole, meropenem, cefoxitin, and cefepime, respectively. However, only few (6) isolates were subjected to Nalidixic acid.

Table 2: Antimicrobial susceptibility of *Vibrio cholerae* (n=279)

Antimcorbial Susceptibility Testing			
Antibiotic type	**Sensitive f(%)**	**Intermediate f(%)**	**Resistance f(%)**
Ampicillin	3(6.0)	(0)	47(94.0)
Ciprofloxacin	73(57.9)	1(0.8)	52(41.3)
Chloramphenicol	79(94.0)	(0)	5(6.0)
Gentamicin	33(86.8)	3(7.9)	2(5.3)
Cefuroxime	53(85.5)	(0)	9(14.5)
Tetracycline	83(90.2)	(0)	9(9.8)
Cefepime	17(37.8)	5(11.1)	23(51.1)
Piperacilin	21(47.7)	2(4.5)	21(47.7)
Amikacin	23(53.5)	6(14.0)	14(32.6)
Cefotaxime	28(56.0)	7(14.0)	15(30.0)
Levofloxacin	32(94.1)	0(0)	2(5.9)
Ceftazidime	14(33.3)		28(66.7)
Erythromycin	5(22.7)		17(77.3)
Trimethoprim-sulfamethoxazole	13(38.2)		21(61.8)
Nalidixic acid	0(0)	0(0)	6(100)
Meropenem	2(28.6)	1(14.3)	4(57.1)
Cefoxitin	3(21.4)	3(21.4)	8(57.1)

Key: f=frequency, % = Percentage

Anti-Microbial Resistance (AMR) of *Vibrio cholerae*

As shown in figure 1 more than two-thirds of the isolates (68.3%) exhibited resistance while 31.7% exhibited resistance to only one antibiotic. The most predominant AMR pattern was Ciprofloxacin + Cefepime (14.6%) followed by Ampicillin + Ciprofloxacin (8.9%) and then Ampicillin + Ciprofloxacin + Nalidixic acid (4.9%). Of the 68.3% isolates that exhibited AMR, 40.7% exhibiting resistance to two antibiotics and 27.6% exhibiting resistance to more than two antibiotics. The predominant pattern of resistance to two antibiotics in the isolates was Ciprofloxacin + Cefepime, followed by Ampicillin + Ciprofloxacin and then Ampicillin + Ceftazidime. For the isolates that exhibited resistance to more than two antibiotics the predominant pattern was Ampicillin + Ciprofloxacin + Nalidixic acid, followed by piperacillin + amikacin + cefoxitin + meropenem, ceftazidime + piperacillin + amikacin + imipenem, piperacillin + cefepime + imipenem, and ceftazidime + piperacillin + amikacin.

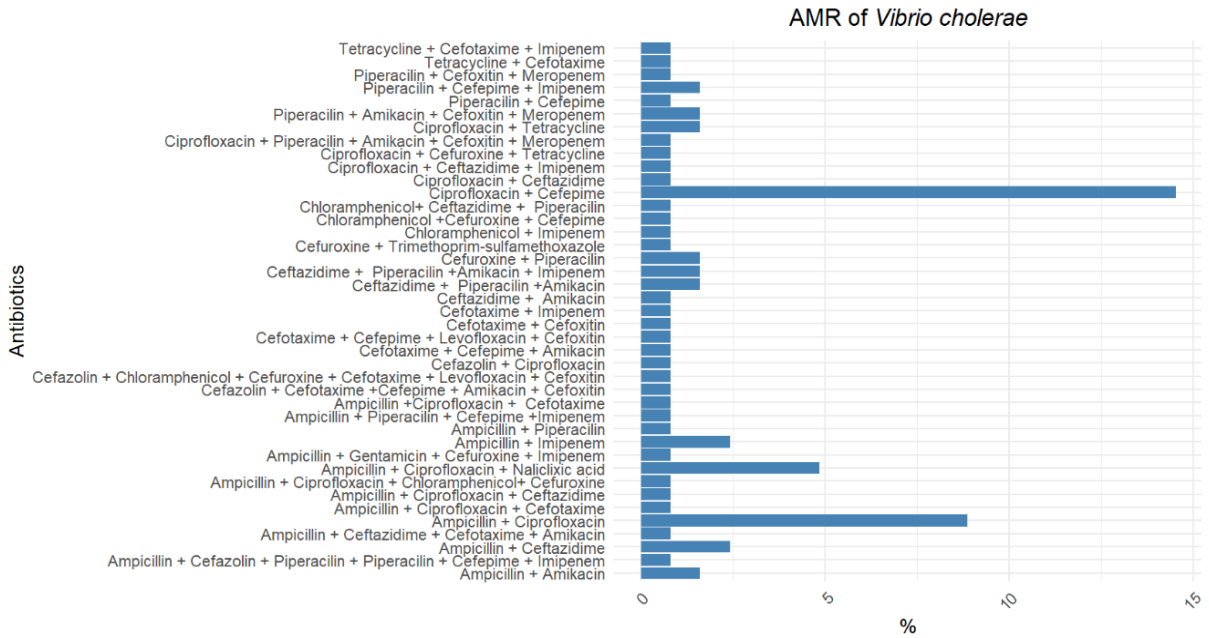

Figure 1: AMR of Vibrio cholerae

Discussion

The study findings indicated that cholera was more common among males. This finding was similar to a study done in Kenya, that investigated cholera epidemics from 2007 to 2010 and 2015 to 2016, which documented that the incidence of cholera was higher in males than in females [18]. This observation could potentially be attributed to men having poor health seeking behavior, high tendency of eating and drinking out and working in unhygienic environments which increases risk of exposure [19,20]. Most isolates demonstrated a high sensitivity to Chloramphenicol, Gentamicin, Cefuroxime, Tetracycline, and Levofloxacin, indicating that these antibiotics are effective treatment options. Isolates from the 2007 to 2010 cholera epidemic in Kenya, cholera outbreaks in Kisumu County in 2014, and cholera outbreaks in Uganda in 2017 showed high sensitivity to Chloramphenicol, Tetracycline, and Gentamicin, respectively [18,21,22]. Furthermore, a Cuban study reported high sensitivity of *V. cholerae* isolates to chloramphenicol (92.0%) and tetracycline (91.2%)[23]. Studies from East Delhi and Mozambique have documented the sensitivity of *V. cholerae* to chloramphenicol (24,25). A meta-analysis of the global antimicrobial status of *V. cholerae* identified Gentamicin and Cefotaxime to be highly effective against *V. cholerae* [16]. Additionally, more than half of the isolates were sensitive to Ciprofloxacin, Amikacin, and Cefotaxime, suggesting emerging resistance to these antibiotics, which may limit their clinical efficacy. Similarly, other studies conducted in Haiti, the United States, and France have reported low resistance of *V. cholerae* to Ciprofloxacin and Cefotaxime [26,28].

The antimicrobial resistance of *V. cholerae* isolates has been increasing in recent years and has become a major risk to cholera treatment strategies[29]. The study revealed high rates of resistance to ampicillin (94.0%), nalidixic acid (100%), erythromycin (77.3%), and ceftazidime (66.7%). The high resistance to first-line antibiotics indicates that these antibiotics are ineffective. Similarly, Kenyan studies reported high resistance of *V. cholerae* to ampicillin (64%), trimethoprim-sulfamethoxazole (54%), and nalidixic acid (100%) [30]. The Ugandan and Cuban Studies also reported 100% and 60% resistance,

respectively, of *V. cholerae* isolates to ampicillin [22,23]. Additionally, isolates from the 2012 to 2015 Mozambique cholera outbreak exhibited 100% resistance to Ampicillin and Nalidixic acid[31]. More than half of the isolates were resistant to trimethoprim–sulfamethoxazole, meropenem, cefoxitin, and cefepime. Similarly, studies in Zambia, Democratic Republic of Congo (DRC) and North India have documented considerable medium resistance of *V. cholerae* to trimethoprim-sulfamethoxazole (SXT) [32,34].

The study findings indicate the levels of resistance, particularly to important classes of antibiotics such as fluoroquinolones (ciprofloxacin and nalidixic acid), extended-spectrum cephalosporins (Cefepime and Ceftazidime), carbapenems (meropenem and imipenem), and penicillins (ampicillin and piperacillin). The high rates of resistance to multiple drug classes, especially carbapenems, which are often considered last-resort antibiotics, are particularly worrying from a clinical perspective. Furthermore, *V. cholerae* resistance to erythromycin is worrying considering that it is safe antibiotic of choice in pregnancy.

In the present study, two thirds (68.3%) of the isolates demonstrated MDR, with the most predominant pattern being Ciprofloxacin + Cefepime. The study findings align with a previous Kenyan study which reported an MDR rate of 64.5% in *V. cholerae* isolates from 2007 to 2010 and 2015–2016 cholera epidemics [18]. Similarly, studies from Ghana and South Africa also highlighted high MDR rates reporting over 97% and 36-50% MDR rates in clinical isolates [38,39]. In contrast a Bangladeshi study conducted among children and older adults documented a lower MDR rate of 28.1%, with the most predominant pattern being TMP-SMX+erythromycin+ampicillin [35].
MDR *V. cholerae* isolates are not a new phenomenon, isolates resistant to Ampicillin, Nalidixic acid, chloramphenicol, and tetracycline began appearing in the 1990s [36]. More recently, a 2021 Kenyan study detected and characterized MDR *V. cholerae* by using genes encoding Extended-Spectrum Beta-Lactamase **(**ESBL) enzymes. ESBL isolates are resistant to chloramphenicol, streptomycin, sulfamethoxazole, trimethoprim, tetracycline, rifampicin, and erythromycin [8]

Evidence increasingly suggests an increase in MDR *V.cholerae* in Kenya and the region [22,37]. The proliferation of MDR among *V.cholerae* isolates as reported by a study in South African may be attributed to spontaneous mutations or horizontal transfer of resistance genes through the co-existence of gut coliforms and *Vibrio species*[40]. This widespread increase in antibiotic resistance majorly influenced by the extensive use of antibiotics has led to the emergence of MDR strains, hence posing a significant global public health concern[18]. Indeed, recent trends indicate that most clinical *V. cholerae* isolates are resistant to almost all routinely used antibiotics [12].

Limitations of the study

Key limitation of this study is the incomplete or inconsistent surveillance data from some counties in Kenya, especially in earlier years, which may compromise the accuracy and reliability of the resistance trend analysis. Additionally, limited geographic coverage, focusing only on selected countries, may restrict the generalizability of the findings to a broader national context. This study also faces the challenge of retrospective design constraints, as it relies on secondary data collected between 2015 and 2023, limiting control over potential confounding variables

and possibly missing emerging resistance patterns.

Conclusion

In conclusion, the antimicrobial resistance profiles of the *V. cholerae* isolates from Kenya have significant clinical implications. Although there were high susceptibility rates to chloramphenicol, gentamicin, cefuroxime, tetracycline, and levofloxacin, alarming resistance to critical and common antibiotics was observed, including complete resistance to nalidix acid and high resistance to ampicillin, erythromycin, and ceftazidime. The *V. cholerae* isolates also exhibited considerably high dual resistance and AMR. The emergence of resistance to carbapenems and the predominant MDR pattern involving ampicillin, ciprofloxacin, and nalidixic acid, which significantly limits treatment options for cholera, indicate the need for enhanced surveillance and development of strategies to address the issue of MDR in cholera management in Kenya.

Recommendation

There is need for evaluation of the observed high resistance of the commonly prescribed antibiotics namely; Nalidixic acid and eryhthromycin among the Cholera patients. Need for strengthening surveillance of Antimircobial Resistance in *V. Cholerae* during Cholera outbreak

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Conflict of interest statement

The author declares that there is no conflict of interest.

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The research was self-funded.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The AMR data for cholera were obtained from National Public Health Laboratory (NPHLS) records, with approval from the Ministry of Health, Kenya. Due to the sensitive nature of the data and ethical considerations, data cannot be shared publicly but may be made available to qualified researchers upon request and with appropriate institutional and ethical approvals.

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