

Systematic Review

Critical evaluation of neglected Consensus risk factors causing the Recurrent Upsurge of Cholera Outbreaks in some regions in Cameroon: A Systematic Review in the context of Etio-epidemiology, socio-political, economic, cultural and environmental determinants of Cholera endemicity, pandemicity or epidemicity in Cameroon.

ABSTRACT

Background: Cameroon is a developing nation and has been enlisted amongst the several countries in sub-Saharan Africa situated in West and Central Africa to be experiencing the recurrent cholera outbreaks. The World Health Organization (WHO), Cameroon health authorities and others researchers have blamed few identified risk factors that are believed to be attributing to the current and on-going cholera outbreaks in Cameroon. These include the wide circulation of *Vibrio cholerae* in the country, limited access to safe-drinking water in some areas, a seasonal pattern of cholera re-occurrence and inadequate water, sanitation and hygienic (WASH) conditions, unstable political atmosphere that have resulted to internally displaced persons, very poor and vulnerable persons, refugees and humanitarian crises and continued unnecessary imposed lock-downs and road-blocks. However, despite these enormous efforts made by Cameroon health authorities so far and its partners over the past decades in responding and tackling all these risk factors, the recurrent outbreak of cholera in Cameroon still remained a great challenge and potential humanitarian crisis, endless reality with bewilderment amid uncertainty and threatening vulnerability to many affected regions.

Aim: This systematic review seeks to critically evaluate, identify and classify some previously overlooked consensus risk factors driving cholera endemicity, pandemicity or epidemicity in Cameroon. By highlighting these neglected factors, this review hopes to inform evidenced-based intervention to finally combat the persistence recurrent upsurge and burden of future outbreaks of cholera in Cameroon.

KEYWORDS: identification, classification, neglected Consensus Risk Factors, Etio-epidemiological, socio-political, economic, cultural and environmental determinants, epidemicity, endemicity, pandemicity, recurrent Cholera outbreaks, Cameroon.

1) INTRODUCTION

A global surge of cholera cases has put one billion people in 43 countries at risk, the World Health Organization (WHO) health authorities have cautioned its members. Researchers have now estimated that each year there are 1.3 to 4.0 million cases of cholera, and 21 000 to 143 000 deaths worldwide due to cholera. Most of those infected have no or mild symptoms and can be successfully treated with oral rehydration solution [1a & 1b]

While cholera is being rapidly eradicated in the developed countries, it still remains a big public health challenges in the developing countries and presently cholera infection is enlisted amongst the neglected tropical diseases [1c]. Historically, cholera can manifest as endemic, epidemic, sporadic or pandemic disease and despite all the major advances in its research, etiology, epidemiology, pathophysiology, pathology, pathogenesis, mode of transmission, diagnosis, treatment and prevention this infectious disease still remains a challenge to the modern medical science in some countries worldwide. Although the disease may be asymptomatic or mild, studies have shown that it can be severe thereby causing elevated or hyper-dehydration state, gastrointestinal disorders, electrolyte derangement and death within hours of onset [2]. Epidemiologically, cholera have been defined as an acute diarrheal infection that is characterized by vomiting, extreme severe depletion of body fluid and salts, caused by ingestion of food or water contaminated with the causative organisms [3, 4]. It has been identified in modern routine and differential cultural media as a Gram-negative bacillus or a bacterium called *Vibrio cholerae* according to the recent conventional microbiological and taxonomical classification [5]. Although more than 200 sero-groups of *V. cholerae* have been

identified, *V. cholerae* O1 and *V. cholerae* O139 are the principal ones associated with epidemic cholera infections and most outbreaks. [9]. *V. cholerae* O1 has caused all recent outbreaks while *V. cholerae* O139 – first identified in Bangladesh in 1992 – caused outbreaks in the past, but recently has only been identified in sporadic cases and it has never been identified outside Asia [10]. There is no difference in the illness caused by the two sero-groups [11]. The short incubation period is between two hours to five days and this explains the exponential rise in cases during outbreaks, leaving a high number of deaths as it spread rapidly everywhere [12]. Recent scanning of electron micrograph (SEM) depicts a number of detailed morphological distinguishing features of *Vibrio cholerae* bacteria of the sero-group O1 from other bacteria [13, 14]. (See figure 1 and figure 2 below)

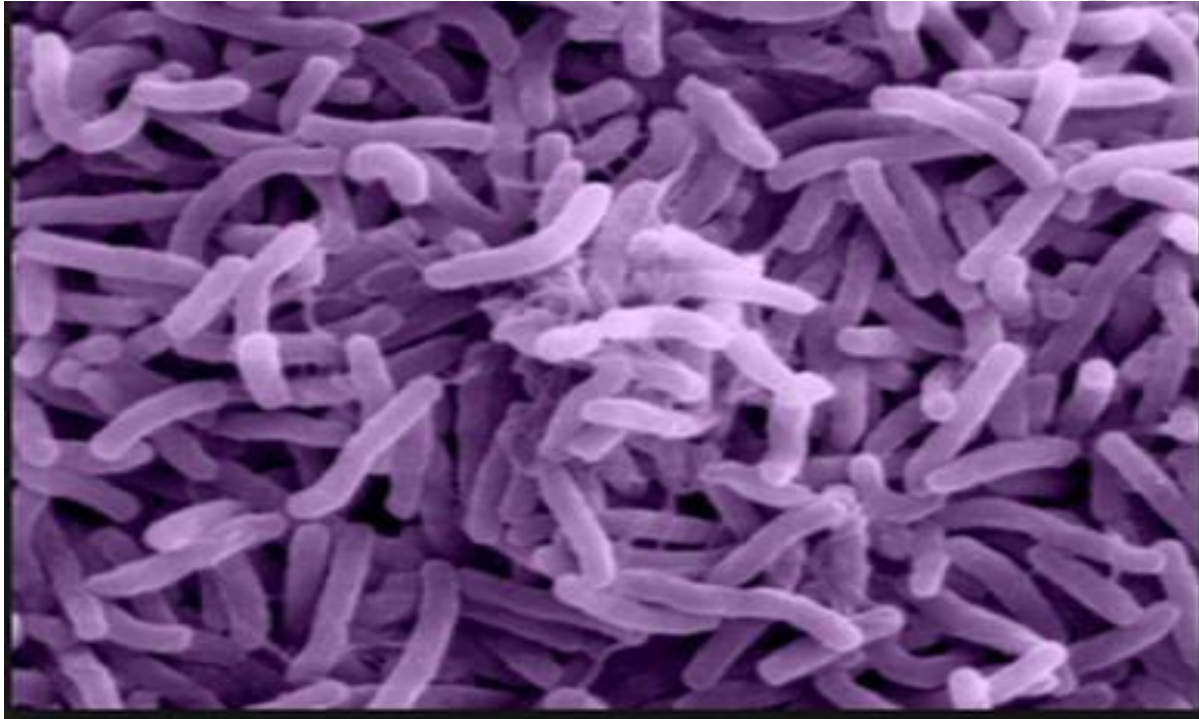


Figure 1: Structure of *V. cholera* bacteria (Source:adopted from Guha, Koushik & Vineetha, K.V. & Sravani, Girija & Sailaja, B.V.s & Kumar, Puli & Kotamraju, Sarat & Prabhakar, VSV & Karumuri, Srinivasa Rao. (2018). Performance Analysis of MEMS sensor for the Detection of Cholera and Diarrhea. Microsystem Technologies, Springer. 24. 10.1007/s00542-018-3810-9.

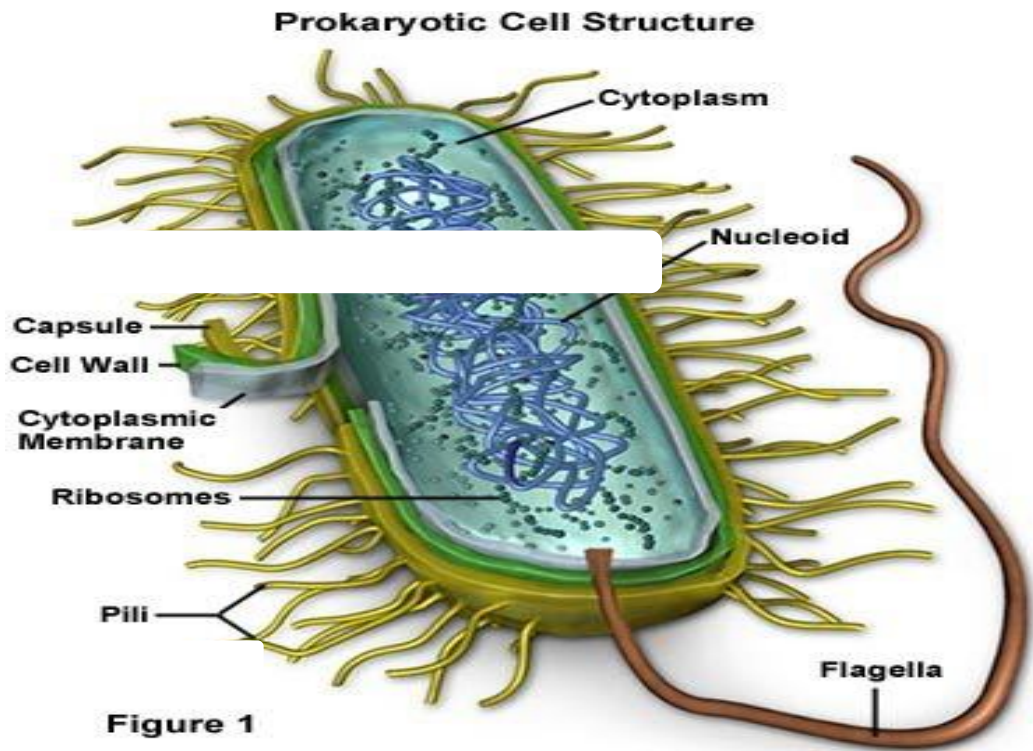


Figure 2: Ultra-modern longitude cross -sectional electronic microscopic structure of *V. cholera* bacteria (Source: adopted from Guha, Koushik & Vineetha, K.V. & Sravani, Girija & Sailaja, B.V.s & Kumar, Puli & Kotamraju, Sarat & Prabhakar, VSV & Karumuri, Srinivasa Rao. (2018). Performance Analysis of MEMS sensor for the Detection of Cholera and Diarrhea. *Microsystem Technologies*, Springer. 24. 10.1007/s00542-018-3810-9.

Microbiological study of the cultural characteristics of *V. cholera* have revealed that the bacterium has a comma-shaped and it is a gram-negative aerobic or facultative anaerobic bacillus that varies in size from 1-3 µm in length by 0.5-0.8 µm in diameter by electronic microscope [15,16]. Its antigenic structure consists of a flagella H antigen and a somatic O antigen and differentiation of the latter allows for separation into pathogenic and nonpathogenic strains [17, 18]. Most cases of *Vibrio cholerae* can be successfully treated with oral rehydration solution (ORS), but severe cases require intravenous fluids and broad-spectrum sensitive antibiotics [19]. Cholera is an extremely virulent disease that can cause severe acute watery diarrhea and the toxicity of its toxins takes between 12 hours and 5 days for a person to show symptoms after ingesting contaminated food or water [2]. Cholera affects both genders and age groups such as infants, children, adults [20] and can kill its victims within hours if left untreated without immediate serious intervening standard medical protocol and remedies [21]. Most people infected with *V. cholerae* do not develop any symptoms, although the bacteria are present in their faeces for 1-10 days after infection and are shed back into the environment, potentially infecting other people [22]. Among people who develop symptoms, the majority have mild or moderate symptoms, while a minority of people develop acute watery diarrhea with severe dehydration which can lead to death if left untreated [23]. While minority of people infected with *V. cholerae* only show mild symptoms, some of them develop severe dehydration that can lead to septic shock, hypovolemic shock and death within hours [33]. Toxicological biomarkers, laboratory findings and Clinical Diagnosis of cholera is confirmed by stool microscopic, culture and sensitivity (use of selective and differential media is recommended and relevant) plus subsequent sero-grouping /subtyping which are available but in reference laboratories; polymerase chain reaction (PCR) testing is also an option. [34, 44]. Point of care Rapid dipstick testing for cholera is available for public health use in areas with limited access to laboratory testing, but specificity of this test is suboptimal, so dipstick-positive specimens should be confirmed by cultures if possible [34] Cholera should be distinguished from clinically similar disease caused by enterotoxin-producing strains of *Escherichia coli* and occasionally by *Salmonella* and *Shigella* species. Biochemical test such as serum electrolytes, blood urea nitrogen, and creatinine should be measured routinely [33].

1.2) PROBLEM STATEMENT OF THE CURRENT REVIEW.

V. cholerae has been considered by the World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) as a global threat to public health and as an indicator of inequity and lack of social development [6, 7]. According to the Global Task force on Cholera Control (GTFCC) 2020, an estimated total of 21,000 to 143,000 deaths worldwide are attributed directly to cholera each year. It is also on record that about 1.3 to 4.0 million of confirmed cases of cholera worldwide are reported each year and approximately each new case is reported every 10 seconds in about 47 countries across the globe. Recent studies have also shown that about 40-80 million people are living in cholera hotspots in Africa alone [8].

Cholera infection in Cameroon have been ongoing and confirmed cases have increased significantly since early 2021 [30]. On October 31, 2021, Cameroon's health authorities declared a cholera outbreak and between October 25 and December 10, 2021, three active regions reported a cumulative number of 309 suspected and four laboratory-confirmed cholera cases, with 19 deaths, for a case fatality ratio (CFR) of 6.1% [31]. Suspected cases increased from 200 recorded on average each week in 2021, to more than 1,262 in the second week of March 2022 and as of April 30, 2022, the WHO had reported 6,652 cases in total [32].

1.3) JUSTIFICATION AND RATIONALE OF THE CURRENT REVIEW

In order to effectively eradicate the continuity of cholera outbreak in affected regions of Cameroon it is very pertinent to investigate or explore, identify and classify all the prevailing consensus risks factors that are enhancing and promoting these endemicities or epidemicities and pandemicities in the affected regions of Cameroon and response appropriately to them thereby uprooting and eradicating the root causes thus providing lasting and eternal solutions.

1.4) REVIEW RESEARCH QUESTIONS

- 1) What are the consensus risk factors (CRF) for another significant outbreak of cholera infection in any region of Cameroon?
- 2) What is the state of preparedness of Cameroon health authorities and her partners for another outbreak of cholera in any region of Cameroon?

1.5) REVIEW HYPOTHESIS (NULL AND ALTERNATIVE HYPOTHESIS)

a) H_0 : There will be no further significant outbreak of cholera infection in any region of Cameroon.

H_A : There will be another significant outbreak of cholera infection in any region of Cameroon.

b) H_0 : The state of preparedness of Cameroon health authorities for another outbreak of cholera in any region of Cameroon is high.

H_A : The state of preparedness of Cameroon health authorities for another outbreak of cholera in any region of Cameroon is low.

1.6) PURPOSE OF THE CURRENT REVIEW

The general purpose of the current review of is to finds out all the reasons, epidemiological identification and classification of consensus risk factors responsible for cholera endemicity, pandemicity or epidemicity in Cameroon.

1.7) SPECIFIC OBJECTIVES

- 1) To make a review of some of the reasons for the continuity and recurrent outbreaks of cholera in Cameroon.
- 2) Tomake a review on the Identification and classification of consensus risk factors responsible for the current outbreaks and circulation of *Vibrio cholera* in Cameroon.

1.8) SIGNIFICANT OF THE CURRENT REVIEW

- 1) The current review is expected to bring to the table all the root causes, identified and classified consensus risk factors responsible for the ongoing and current outbreaks in Cameroon.
- 2) This current review is expected to activate and challenge and evaluate the level of preparedness of the Cameroon health authorities and her partners for another outbreak of cholera in Cameroon.
- 3) The current review is hope to contribute knowledge to the scientific and research community and humanity as a whole

2) REVIEW DESIGNED AND SETTING

a) CASE STUDY COUNTRY

The case study country of the current review is Cameroon which is officially known as the Republic of Cameroon and constitutes one of the developing countries in West-Central Africa being bordered by six neighboring countries namely: Nigeria to the west and north; Chad to the northeast; the Central African Republic to the east; and Equatorial Guinea, Gabon and the Republic of the Congo to the south [24]. Its coastline lies on the Bight of Biafra, part of the Gulf of Guinea and the Atlantic Ocean. Due to its strategic position at the crossroads between West Africa and Central Africa, it has been categorized as being in both camps. Two of Cameroon's border regions with Nigeria (Northwest and Southwest) are Anglophone, while the rest of the country is Francophone with nearly 25 million of its people speaking about 250 native languages [25]. According to the World Bank in Cameroon Report, Cameroon is a lower-middle-income country with a population of over 25 million (2022) estimation [26]. It is partitioned into ten administrative regions which are further divided into 58 districts, 360 sub-districts and 339 councils [27]. In accordance with Decree No. 2013/093 of 3 April 2013 to organize the Ministry of Public Health, the latter is responsible for the formulation and implementation of the Government's public health policies [28]. The health sector in Cameroon is structured into three levels thus forming a pyramidal structure whose functional relationships are

specified and enshrined in the Ministry of the Public Health of Cameroon mandate. It has three sub-sectors namely : a public sub-sector, private sub-sector and traditional sub-sector [28].The public health sector of the country is pyramidal, and has a centralized system of administration that runs from the central (Ministry), through the intermediary (Regional delegations), and cumulating at the peripheral (Health districts) levels. Three different levels of health care delivery services exist in Cameroon; the tertiary, the secondary and the primary services. However, intra-regional differences in health personnel availability which may be associated with urban/rural divide and corresponding economic disparities, could not be assessed due to lack of district-level data [29].

c) MAP OF CASE STUDY AREA

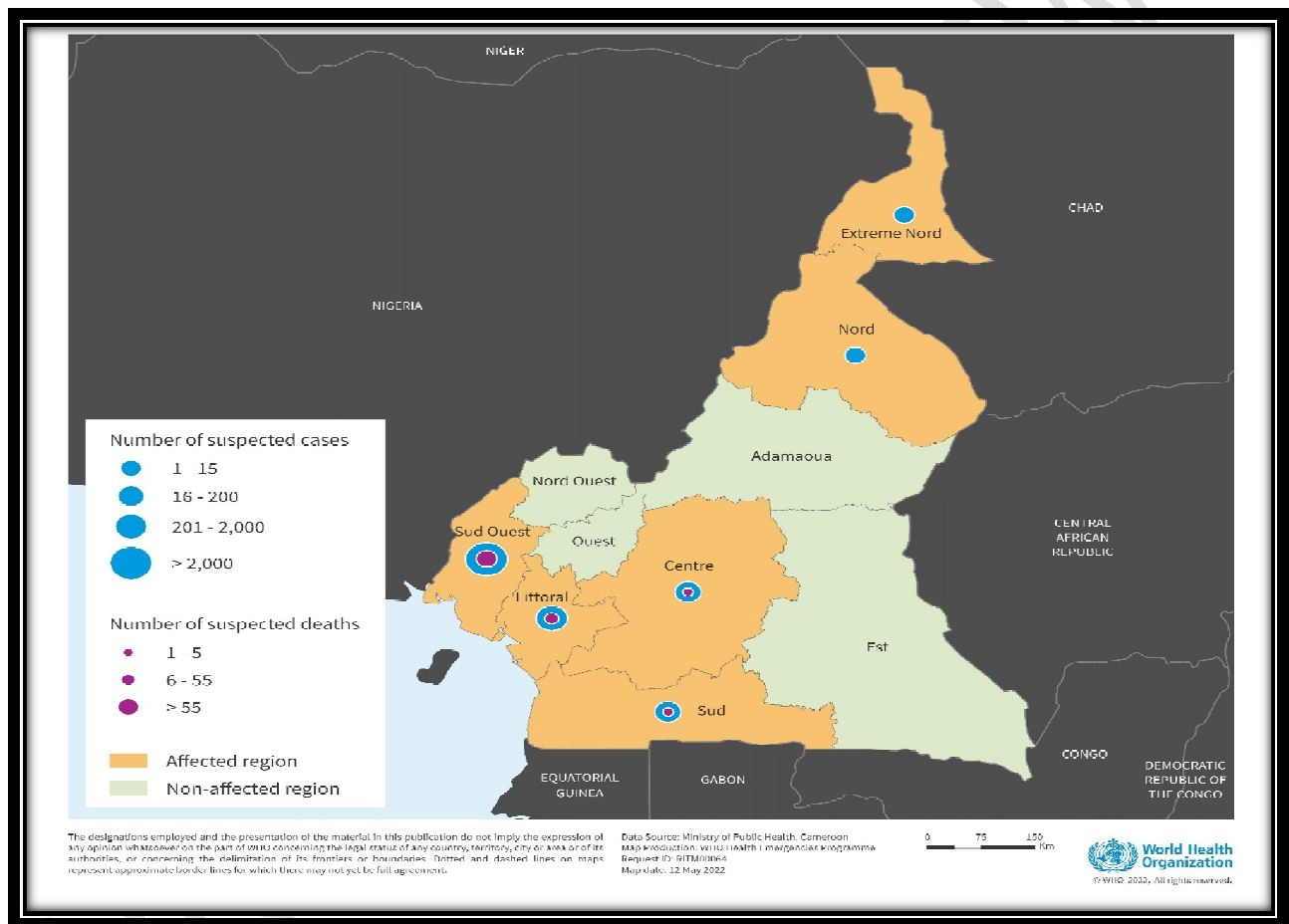


Figure 3: Map of Cameroon showing the number of suspected cases of *Vibrio cholerae* infection and the number of suspected cases of death caused by infection of *Vbrio cholerae* and the affected region and uninfected regions (All Data Source: adopted from the Ministry of Public Health Cameroon and map production by the World Health Organization Emergency Program, Map date: 12th May, 2022, Request ID: RITM00064.

4) RESULTS

Review on the identification and classification of consensus risk factors and some contributing reasons that are responsible for the current outbreaks and circulation of vibrio cholera in Cameroon is summarized in table 1 to 6 below. Each table contains the consensus variables as well as information on the level of intervention and preventive measures taken by the ministry of the public health and WHO and other health partners and stakeholders. There may

be many reasons responsible for the current outbreaks of cholera in Cameroon, some of which have not yet been exploited by other researchers and Cameroon health authorities. The following are some of the several and consensus risk factors to the continuity and circulation of *Vibrio cholera* and outbreaks of cholera in Cameroon especially in the North, Far North, Littoral, Central, South-West and the Western regions of Cameroon [35]. Some of these factors include:

Table 1: classification and identification of consensus environmental risk factors responsible for cholera outbreak in Cameroon

Consensus environmental risk factors variables	Information on Intervention level / preventive measures	Documented Reports and references
1) Warm air temperature,	low	[35].
2) Increased evaporation and water temperature	low	
3) Decreased water levels in rivers	low	
4) The primary source of water for household use in the region	Average	

Table 2: classification and identification of consensus physical and natural risk factors responsible for cholera outbreak in Cameroon

Consensus physical and natural risk factors variables	Information on Intervention level / preventive measures	Documented Reports and references
1) Easy border crossing	High	
2) Man-made disasters such as civil unrest or war that causes disruption of water and sanitation system.	High	[25, 36,37]
3) Natural disasters such as flooding and earth quakes	High	
4) Seasonal changes in geographical and climatic conditions in Cameroon	High	

Table 3: classification and identification of consensus political risk factors responsible for cholera outbreak in Cameroon

Consensus political risk factors variables	Information on Intervention level / preventive measures	Documented Reports and references
1) The internal crises caused by Boko Haram crisis in the North and North far regions of Cameroon	Resolution Ongoing	

2)	The Anglophone crisis in the North and Southwest Regions of the country.	Mixed results	[38, 39, 40,41]
3)	Insecurities cause by the presence of non-state armed men and military presence	Ongoing Little effort	
4)	Unnecessary lock down and shutdown in cities and towns	Little effort	
5)	Road blocks and ghost town imposed by certain non-state armed groups	Little effort	

Table 4: classification and identification of consensus etio-epidemiological risk factors responsible for cholera outbreak in Cameroon

Consensus etio-epidemiological risk factors variables		Information on Intervention level / preventive measures	Documented Reports and references
1)	The epidemiological pattern of cholera outbreak in Cameroon has been proven to be epidemic, endemic, pandemic or sporadic.	Low	
2)	Preparation plan and delay vaccination strategic by intervening groups	Low	[42].
3)	Health system due to lack of basic equipment and testing kits	Low	
4)	Human resources and very high Doctor to patient ratio in Cameroon	Low	
5)	Knowledge of existence of cholera diseases and outbreaks	Low	
6)	Some people in Cameroon do not believe in the existence of cholera and its outbreaks due traditional and cultural practices.	Low	
7)	reported and communication skills about cholera.	Low	
8)	There is low aggressiveness and underreport about cholera to the general public	Low	

Table 5: classification and identification of consensus social risk factors responsible for cholera outbreak in Cameroon

Consensus social risk factors variables		Information on Intervention level / preventive measures	Documented Reports and references
1)	Urban rural migration	High	
2)	Population movement of internally displaced persons (IDPS)	High	[45, 46].
3)	Poor and vulnerable persons (PV)	High	
4)	underreported and poor communication skills about cholera	low	
5)	There low aggressiveness and underreport about cholera to the general public	Low	
6)	Overcrowding camps and lack of social amenities	High	
7)	Shortage and unavailability of safe drinking water	High	
8)	Cultural practices that contribute to unsafe WASH conditions still exist in Cameroon	High	

Table 6: classification and identification of consensus economic risk factors responsible for cholera outbreak in Cameroon

Consensus economic risk factors variable	Information on Intervention level / preventive measures	Documented Reports and references
1) High level of poverty and underdevelopment	Low	
2) limited resources for prevention and care activities in the wide geographical areas involved	low	[43, 44].
3) Insufficient capacity in treatment centers due to overcrowding	low	
4) An insufficient number of cholera treatment kits and medical supplies, such as rapid diagnostic tests, lack of cholera beds and other equipment and poor control of patient flow	low	
5) Inadequate communication tools	Low	
6) Inadequate preparation plan and vaccination strategic	Low	

5.0) DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1) DISCUSSION

Table 1: Shows the identification and classification of consensus of environmental risk factors responsible for cholera outbreak in Cameroon. This consensus of environmental risk factors is directly linked to recurrent cholera outbreaks in Cameroon and include the following: warm air temperature, increased evaporation and water temperature and decreased water levels in rivers, the primary source of water for household use in the region. These factors are in line with those pointed out by other researchers such as [35].

Table 2: Shows the classification and identification of consensus of physical and natural risk factors responsible for cholera outbreak in Cameroon involving the level of response and intervention, and the level of non-response and intervention. These factors include the topographical and geographical features of Cameroon, the natural vulnerability of Cameroon as it lies in the cholera belt. Easy border crossing. Land boundaries of Cameroon: total: 5,018 km, border countries (6): Central African Republic 901 km; Chad 1,116 km; Republic of the Congo 494 km; Equatorial Guinea 183 km; Gabon 349 km; Nigeria 1975 km. [25, 36]. Also, man-made disasters such as civil unrest or war that are causing direct disruption of water and sanitation system. Natural disasters such as floods and earth quakes, seasonal changes in geographical and climatic conditions in Cameroon [37].

Table 3: Classification and identification of consensus political risk factors responsible for cholera outbreak in Cameroon, the level of response and intervention so far and the failure level of non-response and intervention in percentage (%). These factors include: The internal crisis caused by Boko Haram crisis in the Far North and North regions of Cameroon [38]. Studies have shown that the Boko Haram (BH) insurgency in Nigeria expanded into the Far North and North regions of Cameroon in May 2014. Violence and insecurity linked to BH and counter-insurgency operations have caused internal and cross-border displacement, deteriorated socio-economic conditions and led to widespread destruction of houses, infrastructure, roads, markets, health and educational facilities. Nearly 1.9 million people, almost half of the population, need assistance in the Far North, where 74% of its population was already living below the poverty line prior to the BH incursion in 2014. The volatile security situation has restricted food access and availability by limiting agricultural activity, decreasing livelihood options, and weakening trade. [39].

Similarly, the security context in the North-West (NW) and South-West regions (SW) remains volatile, marked by continuous violence, abductions, kidnappings, killings, unlawful arrests, and burning and destruction of properties like villages, houses, hospitals, schools and churches. This aggravates humanitarian needs, has greatly affected people and they continue to flee their homes, seeking safety in the bushes and neighboring communities. Humanitarian access has been negatively affected by several incidents. It is on record that a truck transporting humanitarian supplies has been shot at and stopped by alleged NSAGs elements. The Anglophone crisis in the Northwest and Southwest Regions of the country [40], insecurities caused by the presence of non-state armed men and military presence, unnecessary lock down and shutdown in cities and towns [41] Road blocks and ghost towns imposed by certain non-state armed groups [39] have further aggravated the cholera upsurge directly or indirectly in one way or the other.

Table 4: Classification and identification of individual variables for consensus etio-epidemiological risk factors responsible for cholera outbreak in Cameroon, the level of response and intervention and the level of non-response and intervention are all low [42]. Some of these factors include 1) The epidemiological pattern of cholera outbreak in Cameroon has been proven to be epidemic, endemic, pandemic or sporadic, 2) Preparation plan and delay vaccination strategic by intervening groups, 3) Health system due to

lack of basic equipment and testing kits,4) Human resources and very high Doctor to patient ratio in Cameroon 5)Knowledge of existence of cholera diseases and outbreaks ,6)Some people in Cameroon do not believe in the existence of cholera and its outbreaks due traditional and cultural practices. 7) reported and communication skills about cholera.8) There is low aggressiveness and underreport about cholera to the general public.

Table 5: Classification and identification of individual variables consensus social risk factors responsible for cholera outbreak in Cameroon, the level of response and intervention level, the failure of non -response and intervention i[45, 46]. These includes the following

1)Urban rural migration High, 2)Population movement of internally displaced persons (IDPS) High, 3)Poor and vulnerable persons (PV) High, 4)underreported and poor communication skills about cholera low, 5) There low aggressiveness and underreport about cholera to the general public Low , 6) Overcrowding camps and lack of social amenities High, 7)Shortage and unavailability of safe drinking water High , 8) Cultural practices that contribute to unsafe WASH conditions still exist in Cameroon high

Table 6: Classification and identification of individual variables of consensus economic risk factors responsible for cholera outbreak in Cameroon, the level of response and intervention level of non -response and intervention [43, 44].1)High level of poverty and underdevelopment Low, 2)limited resources for prevention and care activities in the wide geographical areas involvedlow ,3)Insufficient capacity in treatment centers due to overcrowdinglow ,4)Aninsufficient number of cholera treatment kits and medical supplies, such as rapid diagnostic tests, lack of cholera beds and other equipment and poor control of patient flow ,low 5)Inadequate communication tools Low, 6)Inadequate preparation plan and vaccination strategic Low..

5.2) SUMMARY OF FACILITATING FACTORS THAT HAVE BEEN CAUSING CHOLERA OUTBREAKS IN CAMEROON

Unsafe health practices and poor public hygiene and sanitation, municipal water supplies,ice block made from municipal water, foods and drinks sold by street vendors,vegetables grown with water containing human waste,raw or undercooked fish and seafood caught in waters polluted with sewage are considered in the list of the primary and secondary facilitating risk factors contributing for the possible and potential recurrent outbreak of cholerae infection in some regions of Cameroon.

5.3) CONCLUSION:

Although, a lot of researchers including the WHO and Cameroon health authorities have identified, documented and blamed several risk factors that could be attributed to the current and on-going cholera outbreaks in Cameroon, however, despite all these identified and documented risk factors and the enormous efforts done over the past decades by the Cameroon health authorities in this direction to tackle the eradication of cholera in Cameroon, unfortunately there is still a continuous and recurrent outbreak of cholera in Cameroon. This review have therefore demonstrated beyond reasonable doubt that recurrent episode of cholerae in the near future in Cameroon is high possible in line with identification and classification of these consensus risk factors.

5.4) RECOMMENDATIONS:

Based on this review the following recommendations can be made

- a) Public education and sensitization in the light of the classification and identification of the individual consensus risk factors and the variable contents responsible for cholera outbreak in Cameroon
- b) Preventive strategic such as primordial, primary, secondary and tertiary prevention and personal hygiene in the context of the classification and identification of the individual consensus risk factors and the variable contents responsible for cholera outbreak in Cameroon
- c) Adequate preparation for another recurrent outbreak episode of *V. cholerae* in any part of the republic of Cameroon should be based on the Classification and identification of the individual consensus risk factors and the variable contents responsible for cholera outbreak in Cameroon,
- d) A permanent multi-disciplinary and multi-professional team of medical personnel should be put in place and ready to be ready be mobilized and activated in the context of the consensus risk factors.
- e) Consensus risk factors which are able to cause the continuity and circulation of *Vibrio cholera*
- f) Based on the consensus risk factors above prediction and control analysis of future recurrent pattern of the outbreaks of cholera in Cameroon especially in the North, Far North, Littoral, Central, South-West and the Western regions of Cameroon can be studied closely for more strategic planning and rapid intervention.

8) AVAILABILITY OF DATA AND MATERIALS: Datasets generated and analyzed in this study are available from the corresponding author on request.

10) CONSENT FOR PUBLICATION: Not applicable.

12) DISCLAIMER (ARTICIAL INTELLIGENCE)

Author(s) hereby declare that No generative AI technologies such as Large Language Models, ChatGPT, COPILOT etc.) and text-to-image generators have been used during the writing or editing of this manuscript .

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