International Scholars Journals

African Journal of Malaria and Tropical Diseases ISSN 2736-173X Vol. 12 (3), pp. 001-007, March, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Molecular and Resistance Profiles of Vibrio cholerae O1 Strains Isolated in Benin, 2020: A Study of Public Health Significance

Yves Eric Denon[1,2*], Désirée Metodakou[1], Mathieu Odoun[1], Alidehou Jerrold Agbankpe[3], Victorien Dougnon[3], Rolande Assogba[1], Pascaline Mevodjo[1], Germaine Whannou[1] and Honoré Bankolé[1,3]

[1]National Health Laboratory, Ministry of Public Health, 01 P. O. Box, 418 Cotonou, Benin.
[2]Beninese Center for Scientific Research and Innovation, Cotonou, Benin.
[3]Research Unit in Applied Microbiology and Pharmacology of Natural Substances, Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P. O. Box, 2009 Cotonou, Benin.

Received 21 July, 2023; Accepted 23 November, 2023

This study aims to carry out a bacteriological characterization and determine the resistance profile of *Vibrio cholerae* O1 strains isolated during the epidemiological season of 2020 in Benin. To achieve this goal, 43 diarrheal stool samples were analyzed. The samples were taken during the epidemic period of 2020. Bacteriological analyses consisted of enrichment of the samples in buffered peptone water followed by culture on SBCT agar. Then the characteristic colonies were subjected to microscopy, biochemical identification (oxidase, seeding and reading of TSI agar and API 20 E gallery), serotyping, and antibiotic sensitivity tests using the diffusion technique in agar medium according Kirby-Bauer method. The median age of the patients included in this study was 25 years (IQR: 15-40) with predominantly female patients. Individuals aged 11 to 25 were the most represented. Of the 43 stool samples analyzed, 22 were culture positive for *V. cholerae* and belonged to serogroup O1. The clinical manifestations observed in patients with cholera were watery diarrhea, vomiting and severe dehydration before admission to hospital. It should be noted that all of *V. cholerae* O1 strains isolated were multidrug resistant with a strong resistance to erythromycin (81.13%), ampicillin (79.96%), chloramphenicol (79.06%), and cotrimoxazole (78.12%).

Key words: Bacteriological analyses, *Vibrio cholerae* O1, antimicrobial resistance, Benin.

INTRODUCTION

Cholera is a very virulent and fatal diarrheal disease caused by ingestion of water or food contaminated with the bacteria *Vibrio cholerae* (Levade et al., 2017). Vibrio strains can grow and remain for a long time in coastal waters polluted by human feces (Gidado et al., 2018). Seven cholera pandemics have been recorded worldwide

*Corresponding author. E-mail: denric2000@gmail.com.

and continue to cause local and regional outbreaks on the African continent (Danso et al., 2020). Cholera is a major public health problem in many sub-Saharan African countries. According to World Health Organization (WHO), in 2014, 190,549 cases and 2,231 deaths were reported. Out of 2231 deaths, 1882 (84.3%) were from Africa (Bwire et al., 2017). Cholera remains endemic in many countries, in Southeast Asia, Africa, Central and South America. Transmission is closely linked to a lack of access to safe drinking water and sanitation facilities (Abana et al., 2019). Strains of vibrios produce an enterotoxin which causes profuse, painless, watery diarrhea associated with vomiting, leading to severe and fatal dehydration if treatment is not administered promptly (Ngandjio et al., 2009). The main treatment for cholera is based on rehydration. Antimicrobials are a useful therapy for decreasing the duration of diarrhea and bacterial stool and therefore reducing the volume of fluid replacement needed for treatment (Ohene et al., 2016). Widely used antimicrobials include doxycycline or tetracycline for adults, cotrimoxazole (trimethoprim = sulfamethoxazole) for children, and furazolidone for pregnant women (Ngandjio et al., 2009). Since the first pandemic that reached West Africa, some outbreaks have been frequently reported across the region (Moore et al., 2018). However, only a few small-scale studies have been performed to investigate the dynamics of recent cholera outbreaks in West Africa. Overall, the cholera epidemics studied in the region exhibited different characteristics in different countries (Moore et al., 2017). In some countries such as Benin, Ghana, Nigeria and Togo, cases of cholera have been reported every year, although their incidence is relatively low (Landoh et al., 2013; Sule et al., 2017; Moore et al., 2018; Danso et al., 2020). However, in Benin there is a glaring absence of epidemiological data on annual cholera epidemics. It is to remedy this situation and to provide recent epidemiological information on cholera that this study was initiated. It aims to carry out a bacteriological characterization and to determine the resistance profile of the strains of *V. cholerae* O1 isolated in 2020.

MATERIALS AND METHODS

Study framework

This study was conducted in the Republic of Benin. It is a coastal country in West Africa, with an area of 114,763 km^2. The annual average temperatures vary from 26 to 28°C. The climate is subequatorial in the south, with a humid tropical transition in the center and dry tropical in the north (Houehanou et al., 2015). The population was estimated at 9,983,884 inhabitants in 2013 (INSAE, 2016) of which 51.2% were women. The population is elderly with a majority of individuals over 15 years of age. The health system is structured in three levels (central, intermediate and peripheral) in a pyramidal manner (Houehanou et al., 2015). Specifically, the bacteriological diagnosis of the samples was carried out in the bacteriology section of the National Public Health Laboratory, Ministry of Public Health, Benin.

Sampling

Stool samples (43) were systematically taken during the study period (May 8 to October 20, 2020). These samples came from all suspected cases of cholera, notified by health centers and departmental health services. Thus, stool samples from each of these cases were taken into sterile jars and sent to the laboratory immediately for analysis. Samples not sent directly to the laboratory for transport were put on Cary Blair medium and sent to the laboratory within 24 h. Each sample was identified and accompanied by a completed notification form. The information on the sheet concerns the socio-demographic and epidemiological information of the patient.

Sample analysis

Isolation and identification of strains of V. cholerae

Isolation of *V. cholerae* began by enriching samples in buffer peptone water (BPW) (pH 8.4) at 37°C for 6 h, and the resulting solution was streaked onto agar with sucrose bile citrate thiosulfate (SBCT). After 24 h of incubation at 37°C, yellow colonies (sucrose fermenting, 2-3 mm in diameter) suspected of being *V. cholerae* were purified on Müeller Hinton agar for 24 h at 37°C. The purified colonies were characterized by Gram stain (Gram negative comma shaped rods), positive oxidase reaction and reaction in triple sugar iron (TSI) agar. Confirmation of the identity of the putative isolates of *V. cholerae* was achieved by seeding and reading the API 20E gallery.

Serogrouping

The previously identified serogroups of *V. cholerae* strains were serologically confirmed by a slide agglutination test using polyvalent antisera specific for *V. cholerae* O1 and O139, followed by a serotype specific monoclonal antibody.

Antimicrobial susceptibility test

All confirmed isolates of *V. cholerae* O1 were tested for antimicrobial susceptibility on Mëuller Hinton agars using the Kirby-Bauer method of diffusion of the following antibiotic discs: tetracycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ampicillin (10 µg), cotrimoxazole (23.75 µg/1.25 µg), amikacin (30 µg), gentamicin (10 µg), erythromycin (15 µg), streptomycin (10 µg), cefuraxime (30 µg), nalidixic acid (30 µg), doxycycline (30 µg), azetromycin (30 µg), flucloxacillin (5 µg), cefotaxime (30 µg), ceftazidime (30 µg) and chloramphenicol (30 µg). The reference strain *Escherichia coli* ATCC 25922 was used as control. The diameters of the inhibition zones measured were interpreted according to CLSI guidelines (CLSI, 2007).

RESULTS

The sex ratio in the sampled patients was 1.39 in favor of the female sex. The median age of this study population was 25 years (IQR: 15-40). Individuals aged 11 to 15 and 21 to 25 were the most represented with a common percentage of 13.94%, followed by individuals aged 16 to 20, 26 to 30 and 36 to 40 with the same percentage of 9.30% (Table 1). Of the 43 stool samples analyzed, 22 were culture positive for *V. cholerae* and serogrouping

Table 1. Distribution of patients sampled by age and sex.

Age class (years)	Sex		Total (%)
	M (%)	F (%)	
0-5	0(0.00)	3(6.98)	3 (6.98)
5-10	2(4.65)	1(2.33)	3 (6.98)
10-15	2(4.65)	4(9.30)	6 (13.94)
15-20	2(4.65)	2(4.65)	4 (9.30)
20-25	3(6.98)	3(6.98)	6 (13.94)
25-30	3(6.98)	1(2.33)	4 (9.30)
30-35	1(2.33)	2(4.65)	3 (6.98)
35-40	1(2.33)	3(6.98)	4 (9.30)
40-45	2(4.65)	1(2.33)	3 (6.98)
45-50	0(0.00)	2(4.65)	2 (4.65)
50-55	0(0.00)	1(2.33)	1 (2.33)
55-60	0(0.00)	2 (4.654)	2 (4.65)
60-65	1(2.33)	0(0.00)	1 (2.33)
65-70	1(2.33)	0(0.00)	1 (2.33)
Total	18(41.86)	25(58.14)	43 (100.00)

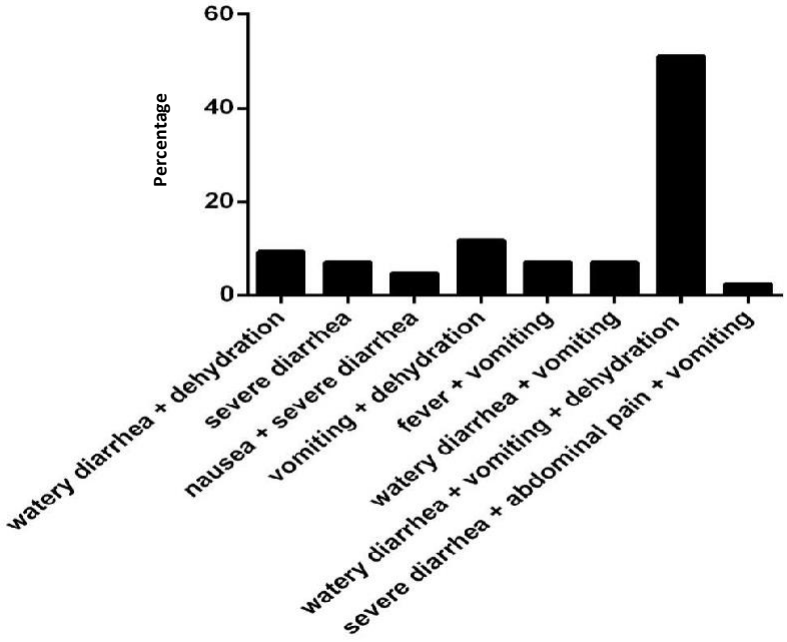

Figure 1. Percentages of different symptoms observed in patients.

resulted in *V. cholerae* O1, that is to say, a prevalence of 51%.

The examination of the notification forms having followed the samples, allowed us to identify 8 symptoms from which the patients suffered before the samples. These symptoms include: watery diarrhea + dehydration; severe diarrhea; nausea + severe diarrhea; vomiting + dehydration; fever + vomiting; watery diarrhea + vomiting; watery diarrhea + vomiting + dehydration and severe diarrhea + abdominal pain + vomiting. The most represented symptoms were watery diarrhea + vomiting + dehydration with 51.16% (n = 22), followed by fever + vomiting (11.63%; n = 05) and watery diarrhea + dehydration (09.30%; n = 04) (Figure 1).

Of the 22 strains of *V. cholerae*, 12 were from samples from female patients and 10 were from samples from male patients: 48% of the samples from female patients and 55.56% of samples from male patients, respectively.

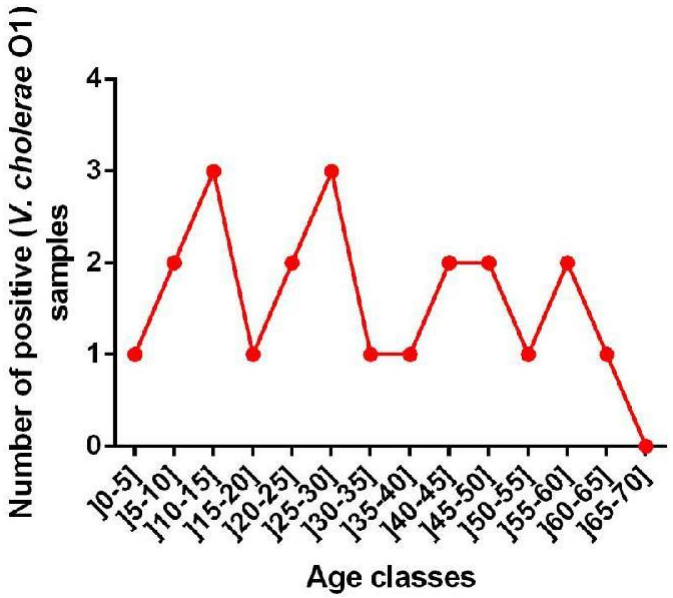

Figure 2. Distribution of positive *V. cholerae* results by age class.

Table 2. Distribution of the analysis results according to the symptoms observed in the patients sampled.

Symptom	Analysis results		Total (%)
	- (%)	+ (%)	
Watery diarrhea + dehydration	04 (19.05)	0	04 (09.30)
Severe diarrhea	03 (14.29)	0	03 (06.98)
Nausea + severe diarrhea	02 (09.52)	0	02 (04.65)
Vomiting + dehydration	05 (23.81)	0	05 (11.63)
Fever + vomiting	03 (14.29)	0	03 (06.98)
Watery diarrhea + vomiting	03 (14.29)	0	03 (06.98)
Watery diarrhea + vomiting + dehydration	0	22 (100.00)	22 (51.16)
Severe diarrhea + abdominal pain + vomiting	01 (4.76)	0	01 (02.33)
Total	21 (48.84)	22 (51.16)	43 (100.00)

Analysis of the data showed an absence of statistical significance ($p > 0.05$) between the positive proportions of the samples in the culture of *V. cholerae* and the sex of the patients from whom these samples were taken.

Of the 22 culture-positive samples for *V. cholerae*, samples from patients aged 11 to 15 and 26 to 30 years old represented the highest proportion with 13.64% each (n = 3) (Figure 2).

Of the 8 symptoms or groups of symptoms observed in patients during sampling, only watery diarrhea + vomiting + dehydration was observed in patients whose specimens tested positive for *V. cholerae* culture (Table 2). Among the culture negative samples of *V. cholerae*, the most prominent symptoms were vomiting + dehydration (23.82%), followed by watery diarrhea + dehydration (19.05%) and severe diarrhea (09.52%). But there was no statistically significant difference ($p > 0.05$) between symptoms and positive culture.

Of the 16 antibiotic discs tested on each of the 22 strains of *V. cholerae*, all strains were susceptible to amikacin, azithromycin, gentamicin, ciprofloxacin, cefuraxime, doxyclyne, cefotaxime and ceftazidine. However, a very strong resistance was noted of the strains isolated to erythromycin (n = 20), to ampicillin (n = 19), to chloramphenicol (n = 18), to cotrimoxazole (n = 17), flucloxacillin (n = 16) and nalidixic acid (n = 16) (Figure 3).

DISCUSSION

The suspected cholera patients included in the study are

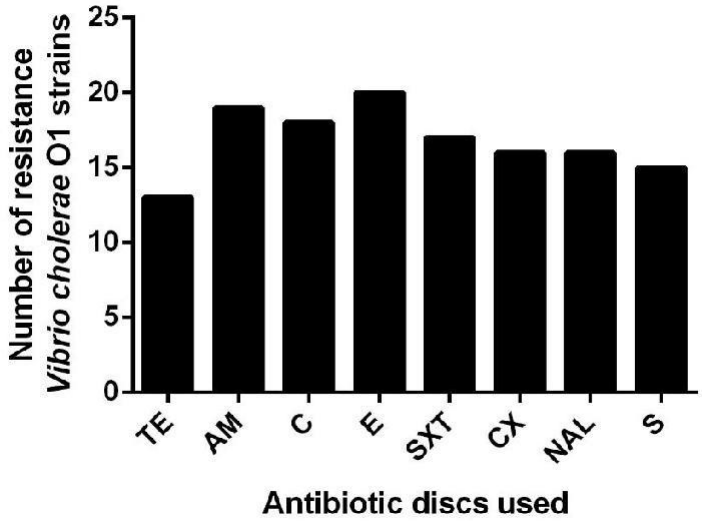

Figure 3. Resistance of *V. cholerae* strains to the antibiotics used. TE: Tétracycline; AM: Ampicillin; C: Chloramphenicol; E: Erythromycin; SXT: Cotrimoxazole; CX: Flucloxacillin; NAL: Nalidixic Acid; S: Streptomycin.

predominantly female, with a median age of 25 years. The most represented age groups were [10-15] and [20-25]. However, statistical analysis of the data has shown that the occurrence of cholera is not related to age or gender. Blacklock et al. (2015) showed that there were more women than men admitted to the Referral Hospital during the cholera epidemic in 2012 in Sierra Leone. As for Danso et al., (2020), they showed that the patients involved in the cholera epidemics in southern Ghana from 2012 to 2015 were mostly men (54.9%), which justify moreover the conclusion according to which the occurrence of cholera in an individual did not depend on sex. However, the fact that in the present study women are more represented than men, could be explained by the exclusive involvement of women in the performance of household chores and the associated risks of cholera transmission. Of the 43 samples analyzed, 22 were declared positive in the culture of *V. cholera* and confirmed to be all strains of *V. cholera* O1, that is, a prevalence of 51%. This prevalence is lower than that found by Koley et al. (2014) 65.6% and that found by Chhotray et al., (2002) in India. All strains of *V. cholerae* isolated in this study are serogroup O1. Both *V. cholerae* serogroups O1 and O139 are involved in cholera epidemics in Africa and globally (Chhotray et al., 2002; Rashed et al., 2012; Lessler et al., 2018). Several studies have shown that in West Africa the *V. cholerae* serogroup most involved in annual cholera epidemics was serogroup O1 (Dalsgaard et al., 1996; Landoh et al., 2013; Moore et al., 2018; Danso et al., 2020). This justifies the results we have obtained. All patients diagnosed positive for *V. cholerae* O1 in the present study were doing watery diarrhea, vomiting and dehydration. The main symptoms of cholera are profuse diarrhea and vomiting, after an incubation period of about 2 h to 5 days (Mengel et al., 2014). Many authors have shown in their studies that the clinical manifestations of patients diagnosed with cholera are diarrhea and vomiting and that on admission to hospital they are severely dehydrated (Ndour et al., 2006; Sule et al., 2017; Elimian et al., 2019). All 22 strains of *V. cholerae* O1 isolated were multidrug resistant with high resistance of the strains to erythromycin, ampicillin, chloramphenicol,

cotrimoxazole (trimethoprim/sulfamethoxazole), flucloxacillin and nalidixic acid. Rashed et al. (2012) also showed that the strains of *V. cholerae* O1 responsible for the 2008 to 2010 cholera epidemic in Bangladesh had high resistance to erythromycin and trimethoprim/ sulfamethoxazole. As for the resistance of the strains to ampicillin and nalidixic acid, Eibach et al., (2016) made the same observations on the strains of *V. cholerae* O1 isolated during the cholera epidemics that occurred in Ghana in 2014. However, the results of the present study showed that all the strains of *V. cholerae* O1 obtained were sensitive to gentamicin, ciprofloxacin, doxyclyne and cefotaxime. The sensitivity of the strains of *V. cholerae* responsible for cholera epidemics to gentamicin, ciprofloxacin and docycline have been reported by several authors (Akoachere et al., 2013; Abana et al., 2019; Danso et al., 2020). Cotrimoxazole (trimethoprim = sulfamethoxazole), formerly first-line treatment in children, was ineffective in 78.12% of isolates. Tetracycline, recommended by the WHO for the treatment of cholera in adults, was found to be effective against 51.8% of the

strains tested and ineffective against 48.2% of the latter. This situation then poses a serious problem of therapeutic failure in the treatment of cholera with antibiotics. However, it should be noted that in the treatment of cholera, rehydration remains the main treatment.

Conclusion

This study confirms that the annual cholera epidemics in Benin are the cause of the same *V. cholerae* serogroup, which is serogroup O1 and has shown that the disease can affect all ages, and 90% of them manifest as watery diarrhea, vomiting, followed by severe dehydration. The results showed an increasing trend of multi-resistant *V. cholerae* O1 strains in Benin. Ciprofloxacin, tetracycline and doxycycline remain effective against clinical strains. But a strong resistance of these same strains to erythromycin, tetracycline, ampicillin and trimethoprim/sulfamethoxazole were noted. The results indicate an urgent need for the appropriate use of antibiotics, hygiene and sanitation of coastal villages in Benin in general to prevent the cholera epidemic.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank all the staff of the National Public Health Laboratory of the Ministry of Health of Benin, especially all the staff of the bacteriology section.

REFERENCES

Abana D, Gyamfi E, Dogbe M, Opoku G, Opare D, Boateng G, Mosi L (2019). Investigating the virulence genes and antibiotic susceptibility patterns of Vibrio cholerae O1 in environmental and clinical isolates in Accra, Ghana. BMC Infectious Diseases 19:76.

Akoachere JFTK, Omam LA, Massalla TN (2013). Assessment of the relationship between bacteriological quality of dug-wells, hygiene behaviour and well characteristics in two cholera endemic localities in Douala, Cameroon. BMC Public Health 13:692.

Clinical and Laboratory Standards Institute (CLSI) (2007). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. M100. 27(1):182.

INSAE (2016). Effectifs de la population des villages et quartiers de ville du benin (rgph-4, 2013), P 85. https://insae.bj/images/docs/insaestatistiques/demographiques/population/Effectifs%20de%20la%20population%20des%20villages%20et%20quartiers%20de%20ville%20du%20benin/Cahier%20Village%20RGPH4%202013.pdf

Blacklock A, Sesay A, Kamara A, Kamara M, Blacklock C (2015). Characteristics and clinical management of patients admitted to cholera wards in a regional referral hospital during the 2012 epidemic in Sierra Leone. Global Health Action 8(1):-25266.

Bwire G, Munier A, Ouedraogo I, Heyerdahl L, Komakech H, Kagirita A, Wood R, Mhlanga R, Njanpop-Lafourcade B, Malimbo M, Makumbi I, Wandawa J, Gessner BD, Orach CG, Mengel MA (2017). Epidemiology of cholera outbreaks and socio-economic characteristics of the communities in the fishing villages of Uganda 2011-2015. PLoS Neglected Tropical Diseases 11:e0005407.

Chhotray GP, Pal BB, Khuntia HK, Chowdhury NR, Chakraborty S, Yamasaki S, Ramamurthy T, Takeda Y, Bhattacharya SK, Nair GB (2002). Incidence and molecular analysis of Vibrio cholerae associated with cholera outbreak subsequent to the super cyclone in Orissa, India. Epidemiology and Infection 128:131-138.

Dalsgaard A, Mortensen HF, Mølbak K, Dias F, Serichantalergs O, Echeverria P (1996). Molecular characterization of Vibrio cholerae O1 strains isolated during cholera outbreaks in Guinea-Bissau. Journal of Clinical Microbiology 34:1189-1192.

Danso EK, Asare P, Otchere ID, Akyeh LM, Asante-Poku A, Aboagye SY, Osei-Wusu S, Opare D, Ntoumi F, Zumla A, Duodu S, Yeboah-Manu D (2020). A molecular and epidemiological study of Vibrio cholerae isolates from cholera outbreaks in southern Ghana. PloS One 15:e0236016.

Eibach D, Herrera-León S, Gil H, Hogan B, Ehlkes L, Adjabeng M, Kreuels B, Nagel M, Opare D, Fobil JN, May J (2016). Molecular Epidemiology and Antibiotic Susceptibility of Vibrio cholerae Associated with a Large Cholera Outbreak in Ghana in 2014. PLoS neglected Tropical Diseases 10:e0004751.

Elimian KO, Musah A, Mezue S, Oyebanji O, Yennan S, Jinadu A, Williams N, Ogunleye A, Fall IS, Yao M, Eteng W-E, Abok P, Popoola M, Chukwuji M, Omar LH, Ekeng E, Balde T, Mamadu I, Adeyemo A, Namara G, Okudo I, Alemu W, Peter C, Ihekweazu C (2019). Descriptive epidemiology of cholera outbreak in Nigeria, January-November, 2018: implications for the global roadmap strategy. BMC Public Health 19:1264.

Gidado S, Awosanya E, Haladu S, Ayanleke HB, Idris S, Mamuda I, Mohammed A, Michael CA, Waziri NE, Nguku P (2018). Cholera outbreak in a naïve rural community in Northern Nigeria: the importance of hand washing with soap, September 2010. The Pan African Medical Journal 30:5.

Houehanou YCN, Lacroix P, Mizehoun GC, Preux PM, Marin B, Houinato DS (2015) Magnitude of Cardiovascular Risk Factors in Rural and Urban Areas in Benin: Findings from a Nationwide Steps Survey. PLoS One 10. https://doi.org/10.1371/journal.pone.0126441

Koley H, Ray N, Chowdhury G, Barman S, Mitra S, Ramamurthy T, Mukhopadhyay AK, Sarkar BL, Katyal R, Das P, Panda S, Ghosh S (2014). Outbreak of cholera caused by Vibrio cholerae O1 El Tor variant strain in Bihar, India. Japanese Journal of Infectious Diseases 67(3):221-226.

Landoh DE, Essoya LD, Gessner BD, Badziklou K, Kossi B, Tamekloe T, Tsidi T, Nassoury DI, Ibrahim ND, Dagnra A, Anoumou D, Patassi A, Akouda P, Tante O, Bidjada B, Tigossou S, Banla KA, Abiba BK (2013). National surveillance data on the epidemiology of cholera in Togo. The Journal of Infectious Diseases 208(Suppl 1):S115-119.

Lessler J, Moore SM, Luquero FJ, McKay HS, Grais R, Henkens M, Mengel M, Dunoyer J, M'bangombe M, Lee EC, Djingarey MH, Sudre B, Bompangue D, Fraser RSM, Abubakar A, Perea W, Legros D, Azman AS (2018). Mapping the burden of cholera in sub-Saharan Africa and implications for control: an analysis of data across geographical scales. Lancet 391(10133):1908-1915.

Levade I, Terrat Y, Leducq J-B, Weil AA, Mayo-Smith LM, Chowdhury F, Khan AI, Boncy J, Buteau J, Ivers LC, Ryan ET, Charles RC, Calderwood SB, Qadri F, Harris JB, LaRocque RC, Shapiro BJ (2017). Vibrio cholerae genomic diversity within and between patients. Microbial Genomics 3(12). https://doi.org/10.1099/mgen.0.000142

Mengel MA, Delrieu I, Heyerdahl L, Gessner BD (2014). Cholera outbreaks in Africa. Current Topics in Microbiology and Immunology 379:117-144. doi: 10.1007/82_2014_369

Moore S, Dongdem AZ, Opare D, Cottavoz P, Fookes M, Sadji AY, Dzotsi E, Dogbe M, Jeddi F, Bidjada B, Piarroux M, Valentin OT, Glèlè CK, Rebaudet S, Sow AG, Constantin de Magny G, Koivogui L, Dunoyer J, Bellet F, Garnotel E, Thomson N, Piarroux R (2018). Dynamics of cholera epidemics from Benin to Mauritania. PLoS Neglected Tropical Diseases 12:e0006379.

Moore SM, Azman AS, Zaitchik BF, Mintz ED, Brunkard J, Legros D, Hill A, McKay H, Luquero FJ, Olson D, Lessler J (2017). El Niño and

the shifting geography of cholera in Africa. Proceedings of the National Academy of Sciences of the United States of America 114(17):4436-4441.

Ndour CT, Manga NM, Ká R, Dia-Badiane NM, Fortez L, Seydi M, Soumaré M, Sow AI, Diop BM, Sow PS (2006). [Cholera epidemic of 2004 in Dakar, Senegal: epidemiologic, clinical and therapeutic aspects]. Medecine Tropicale: Revue Du Corps De Sante Colonial 66(1):33-38.

Ngandjio A, Tejiokem M, Wouafo M, Ndome I, Yonga M, Guenole A, Lemee L, Quilici ML, Fonkoua MC (2009). Antimicrobial resistance and molecular characterization of Vibrio cholerae O1 during the 2004 and 2005 outbreak of cholera in Cameroon. Foodborne Pathogens and Disease 6(1):49-56.

Ohene SA, Klenyuie W, Sarpeh M (2016). Assessment of the response to cholera outbreaks in two districts in Ghana. Infectious diseases of poverty 5(1):1-11.

Rashed SM, Mannan SB, Johura FT, Islam MT, Sadique A, Watanabe H, Sack RB, Huq A, Colwell RR, Cravioto A, Alam M (2012). Genetic characteristics of drug-resistant Vibrio cholerae O1 causing endemic cholera in Dhaka, 2006-2011. Journal of Medical Microbiology 61(12):1736-1745.

Sule IB, Yahaya M, Aisha AA, Zainab AD, Ummulkhulthum B, Nguku P (2017). Descriptive epidemiology of a cholera outbreak in Kaduna State, Northwest Nigeria, 2014. The Pan African Medical Journal 27:172.