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# Molecular Epidemiology of *Vibrio cholerae* Recovered from Sewage Drains, Captured Fish and Humans in 2015/16 Cholera Outbreak in Zanzibar, Tanzania

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***Authors' contributions***

*This was a collaborative work among all authors. Author ARR designed the study, collected samples from field, performed the laboratory work and wrote the first draft of the manuscript. Authors SIK, PNW, GM and RHM assisted on study design and in drafting the laboratory protocol organizing and refining the manuscript. Author FAK collected specimen from human patients, did laboratory work and went through the manuscript. Author AM contributed on literature citing, organizing and fine tuning of the manuscript according to journal requirements. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Zanzibar shares global burden of cholera epidemic suffering annual outbreaks with case fatality rates (CFR) of 1.8%. This study aimed at determining the transmission chain of the outbreak through marine fish by molecular characterization of *Vibrio cholerae* isolates.
**Study Design:** Cross sectional design was used to collect samples from fish, sewage sites and stool from clinical cases.
**Place and Duration of Study:** The study was carried out in Zanzibar municipality between November 2015 and May 2016.
**Methodology:** Epidemiological data on the outbreak was provided by the Ministry of Health and Social Affairs, Zanzibar. Sixty samples from fish intestines, 23 samples from sewage drains and 38 human stools were investigated. *Vibrio cholerae* was identified biochemically and serology was done using Polyvalent, Inaba, Ogawa and O139 antisera. Kirby-Bauer disc diffusion was used for antibiotic sensitivity against seven antibiotics. DNA was extracted and PCR performed using *ctxB* forward and reverse *ctxB* primers. Gene sequences were interpreted by Mega 7.0 software.
**Results:** Thirty stool samples (79%), 9 fish samples (15%) and 6 sewage samples (26%) were positive for *Vibrio cholerae*. All isolates were identified: serogroup O1, biotype El Tor and serotype Ogawa. Age category 16-30 yrs old had highest number of cases (37.6%). Case fatality rate (CFR) was 1.8%, more mortalities were in males and >5yrs old. None of the isolates was resistant to ciprofloxacin. High resistance was against nalidixic acid, erythromycin, co-trimaxozole, ampicillin and tetracycline. Multidrug resistance was observed in 40% of isolates. *CtxB* gene sequencing revealed that the current *Vibrio cholerae* strain was homologous to Haiti 2013-2015 and China 2016 strains, but distinct from Zanzibar 2013 strain.
**Conclusion:** Fish could be the source of *Vibrio cholerae* transmission in Zanzibar. Presence of rapidly emerging endemic reservoir of *Vibrio cholerae* in Zanzibar environment was suspected favouring horizontal gene transfer with resultant novel strains. High drug resistance and multidrug resistance are matters of public health concern.


*Keywords: Fish; sewage drains; Vibrio cholera; ctxB gene; Zanzibar.*

## 1. INTRODUCTION

*Vibrio cholerae* is a genetically versatile gram negative curved rod bacterium responsible for pandemic clinical syndrome characterised by painless, watery usually voluminous diarrhea, transmitted through faecal-oral route [1,2]. The infection is related to sanitation and hygiene and mostly associated with water and food contamination in dirty human settings [1,3,4]. Cholera outbreaks occur in seasonal pattern and more common in rainfall seasons [5,6].

Seven global cholera pandemics have occurred from 1816 up to 1991 [7,8]. An estimated 1.4 billion people globally are at risk of cholera infection, with 3-5 million cases and annual mortality of 100,000 to 120,000, majority of whom are under five years old [9]. The disease, even though less reported in developed countries, is still prevalent in many parts of the world including sub-Saharan Africa, Indian sub-continent and Latin and Central American countries [5,10,11]. Africa south of Sahara account for 60% of the global burden of cholera cases while South East Asia account for 29% [12]. However gross under reporting exist worldwide and WHO estimate that only 5-10% of the cases occurring annually are officially reported [13].

The first cholera cases in Tanzania were reported in 1974 and since then cholera has been endemic with case fatality rate of 10.5% [14]. Zanzibar had its first major cholera outbreak in 1978 and since then it has been occurring almost annually [15].

*Vibrio cholerae* is waterborne and a normal inhabitant of esturine and riverine waters. Variety of zooplankton, phytoplankton, and algae enable the bacterium survive harsh conditions in a viable but non-culturable state [16,17,18]. As a result sea foods including oysters and fish that, in most of the cases consume these organisms have been implicated as sources of *Vibrio cholerae* to humans [19,20,21]. Toxigenic and non-toxigenic *Vibrio cholerae* isolates co-exist in the environment, a phenomenon that encourages exchange of genetic material through horizontal gene transfer [17,22]. Among factors necessary for bacterium to acquire virulence and epidemic

nature are the aquatic environment, intestinal environment of the host population, presence of CTXΦ lytic phage component, mobile genetic elements and other genes like toxin-co-regulated pilus (TCP) which is involved in the acquisition of virulence genes through horizontal gene transfer [23,24,6,25].

*Vibrio cholerae* has a capacity of resorting to biofilm state outside the host [26,27] and has mechanisms of acquiring external genetic material from phages as well as after deliberate killing of other cells [18,28]. More than 200 *Vibrio cholerae* serogroups are known to exist out of which serogroup O1 and O139 are most commonly associated with epidemic cholera [29]. Serogroup O1 has two biotypes, Classical and El Tor, responsible for the first six outbreaks and seventh global pandemics respectively [23]. Both biotypes are further divided into serotypes Inaba, Ogawa and Hikojima [30]. *Vibrio cholerae* serogroup O139, potentially could cause 8$^{th}$ global pandemic, was discovered in 1992 in Bangladesh and is now endemic in at least eleven countries [31].

Clinical symptoms of *Vibrio cholerae* are due to possession of potent enterotoxin, CT, which promotes effusion of fluid into intestinal lumen. The CT is coded by *ctxAB* genes present in CTXØ genome which is derived from lytic phages [32,33]. The capacity to acquire external genomes and mutations contribute to evolution of new genotypes of *Vibrio cholerae* with its accompanying change in epidemiology, pathogenicity and drug sensitivity [6,18,33,34].

Antibiotic drug resistance in *Vibrio cholerae* is well documented worldwide and it is a serious problem especially in cholera endemic countries [35-38]. Drug resistance in *Vibrio cholerae* has been found against ampicillin, cotrimoxazole, tetracycline, sulfamethaxazole, trimethoprim, nalidixic acid and azithromycin [39]. Multidrug resistance (MDR) in *Vibrio cholerae,* which is resistance to more three classes of antibiotics [40] is also a global problem [35,41]. So far there is scanty information on drug resistance in *Vibrio cholerae* in Zanzibar and Tanzania as a whole.

The aim of this study was to investigate if fish could be source of transmission of *Vibrio cholerae* to humans and determine the serology, drug sensitivity, genetic lineage and epidemiology of the *Vibrio cholerae* isolated from three sources- fish, environment and stools of clinical human patients in Zanzibar during the 2015/16 outbreak.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This study was carried out in Zanzibar municipality where cholera outbreak started. Sites under investigation were Mnazimmoja hospital, sewage drains in the town area and marine fish captured close to sewage draining sites.

### 2.2 Research Design

Cross sectional design was used to collect all samples. Samples were collected during period of outbreak between November 2015 and May 2016.

### 2.3 Sample Collection, Bacterial Isolation and Identification

Following cholera outbreak in Zanzibar between September 2015 and July 2016 stool samples from 38 patients were collected in sterile containers and sent to Mnazimmoja hospital laboratory for bacterial isolation and confirmation by traditional microbiological methods and serology. Twenty three samples from sewage drains in Zanzibar municipality and 60 samples from sea fish intestines were analysed at the Zanzibar Central Veterinary laboratory, making the total number of 121 samples.

In the laboratory 25 ml of sewage water/25 g of faecal samples were mixed, for enrichment, with 225 mls of Alkaline Peptone Water-APW (Sigma, Steinheim, Germany) and incubated at 37°C overnight. For fish samples 5g were mixed with 45mls of APW. Samples were then sub-cultured in Thiosulfate Citrate Bile Sucrose (TCBS) agar (Oxoid, England) for 24hrs. Suspected *Vibrio cholerae* yellow colonies were re-sub-cultured in Blood Agar (Oxoid, England) enriched with 5% human blood. Oxidase test using oxidase strips (Oxoid, England) and serology were used for confirmation. Isolates were tested with antisera-Polyvalent, Inaba, Ogawa and O139 (Denka Seiken, Japan).

Biotype identification was determined by haemolysis of sheep erythrocytes and the Voges-Proskauer test, which measured the production of acetylmethylcarbinol.

#### 2.3.1 Antibiotic susceptibility test

Kirby-Bauer disc diffusion method was used for antibiotic sensitivity tests. Pure overnight cultures of *Vibrio cholerae* isolates were mixed with sterile saline and, after matching with 0.5 McFarland Standard, were inoculated in Muller-Hinton Agar (OXOID, England). Antibiotic discs (OXOID, England) were impregnated on to the Agar. *Vibrio cholerae* isolates (30 from human stool, 5 from sewage water and 11 from fish) were tested against ampicillin (AMP, 10 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), co-trimoxazole (COT, 25µg µg), nalidixic acid (NAL 30 µg) and tetracycline (TE, 20 µg). Inhibition diameter zone readings were recorded according to Clinical and Laboratory Standards Institute (CLSI) 2012 [42] standard breakpoints. *Escherichia coli* ATCC25922 was used as reference strain for quality control.

#### 2.3.2 DNA extraction, PCR amplification

DNA from pure colonies of isolated bacteria was done at Sokoine university of Agriculture, College of Veterinary Medicine and Biomedical Sciences (CVMBS), Molecular biology laboratory was extracted using QiaAmp nucleic extraction kits (Qiagen, Hilden, Germany) following the manufacturer's instructions. Pure colonies of isolated bacteria was picked using a sterile pipette tip and mixed with sterile distilled water. Bacteria were incubated with 20 µl of 20 mg/ml proteinase K (Macherey-Nagel, Düren, Germany) at 55 °C for 2 hour. After DNA extraction PCR was performed using *ctxB* forward (5'-GGT TGC TTC TCA TCA TCG AAC CAC -3') and *ctxB* reverse (5'-GAT ACA CAT AAT AGA ATT AAG GAT G -3') primers that amplify *Vibrio cholerae* toxin B subunit gene (*ctxB*) using DNA polymerase (Thermo scientific PCR mix, USA). The expected size of a PCR products fragment ware around 460 bp segment of *ctxB* (Figure not shown). PCR amplification conditions included an initial denaturation at 95°C for 10 minutes followed by 25 cycles of denaturation 95 °C for 1 minutes, annealing 55°C for 1 minutes and extension 72°C for 1 minutes and a final extension at 72°C for 10 minutes using GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). Afterwards, PCR products were electrophoresed in a 1.5% agarose gel mixed with GelRed nucleic acid stain (Phenix Research Products, Candler, NC) before visualization and imaging using a gel documentation system (GelDoc-EZ Imager, Bio-Rad Laboratories, USA).

#### 2.3.3 Sequencing

In order to verify the retrieval of fragments the bacteria of interest, PCR fragments were purified from agarose gels using a NucleoSpin gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany) and subjected to dideoxynucleotide cycle sequencing by using Big Dye Terminator Cycle Sequencing Kit Version 3.1 (Applied Biosystems, Foster City, CA). Products from dideoxynucleotide cycle sequencing reaction were purified by ethanol precipitation and separated on a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA).The sequences from this study were blasted in the NCBI to obtain reference sequences that were interpreted together by phylogeny in Mega 7.0 software.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Culture and isolation

Total of 38 diarrheic stool samples, 60 fish samples and 23 sewage samples were sent to laboratory for bacterial isolation and identification. Thirty stool samples (79%), 9 fish samples (15%) and 6 environment samples (sewage drainage) (26%) were positive for *Vibrio cholerae*.

#### 3.1.2 Biotypes identification

All the 45 isolates were identified to be El Tor biotype.

#### 3.1.3 Serology

All 45 isolates were positive for Polyvalent and Ogawa antisera and negative for Inaba and O139 antisera confirming all cholera isolates in the current outbreak were serogroup O1, biotype El Tor and serotype Ogawa.

#### 3.1.4 Epidemiological description of 2015/2016 outbreak

Outbreak started on September 2015 in the Zanzibar town and when reached July 2016 cholera had spread throughout Zanzibar Island with total cases of 2652 and death toll of 48 while Case Fatality Rate (CFR) was 1.81%. Table1 illustrate age and gender distribution of infected cases while Table 2 depict distribution of mortalities. Age groups 16-30 yrs, irrespective of

**Table 1. Age and gender distribution of reported cholera cases**

| | Reported cases (%) | | | | | |
|---|---|---|---|---|---|---|
| **Age (yrs)** | 0-5 | 6-15 | 16-30 | 31-50 | >51 | Total |
| **Gender** | | | | | | |
| Male | 220 (15.8) | 306 (22.0) | 526 (37.8) | 231 (16.6) | 109 (7.8) | 1392 |
| Female | 150 (11.9) | 209 (16.6) | 472 (37.5) | 283 (22.5) | 146 (11.6) | 1260 |
| **Total** | 370 (14.0) | 515 (19.4) | 998 (37.6) | 514 (19.4) | 255 (9.6) | 2652 |

gender, had highest number of cases (998; 37.6%) and age group >51 yrs had least number of cases (255; 9.6%). Males were more affected than females. Table 2 shows that males had higher mortalities (62.5%) than females (37.5%) and more deaths were reported in above 5yrs group (63.5%).

**Table 2. Mortalities of clinical cases**

| | Mortality cases (%) | |
|---|---|---|
| Age (yrs) | Under 5 | Above 5 |
| | 18 (37.5) | 30 (62.5) |
| Gender | Male | Female |
| | 30 (62.5) | 18 (37.5) |

#### 3.1.5 Antibiotic susceptibility pattern of *Vibrio cholerae* isolates

A panel of seven antibiotics of different classes was used to test susceptibility of isolates and therefore recommend drugs to be used for empirical therapy. Results in Table 3 show that all isolates were susceptible to Ciprofloxacin. The next drug that followed on sensitivity was Chloramphenicol (87%) while nalidixic acid (7%). had lowest sensitivity.

Nine antibiotic patterns were observed (Table 4). Multidrug resistance (MDR), as defined by Awasthi et al. [40] and Magiorakos et al. [43], meaning resistance to three or more classes of antibiotics, was observed in 18 strains (40.0%).

#### 3.1.6 Phylogenetic tree-*ctxB* gene

The phylogenetic tree obtained by *ctxB* gene sequencing (Fig. 1) indicated that all 4 *Vibrio cholerae* isolates in 2017; Z1 from fish, Z2 from sewage, and Z3, and Z4 from patients belonged to one cluster to which Haiti isolates of 2013, 2014 and 2015 outbreaks also belong. China 2016 isolate belong to the same cluster as well. The Zanzibar 2017 isolate also differ markedly with isolates from Russia, Australia, Asia, India, Japan and Zanzibar 2013 isolates. Table 5 enlist the isolates referred in the phylogenetic tree with their accession numbers.

### 3.2 Discussion

This study investigated if fish foods could be source of *Vibrio cholerae* infection in Zanzibar population in the Zanzibar 2015/16 cholera outbreak. This study also provided information on prevalent *Vibrio cholerae* serotypes, epidemiological information of the cholera outbreak, drug sensitivity patterns and molecular characterisation of the *Vibrio cholerae* isolates using *ctxB* gene.

The current study confirmed that the 2015/16 cholera outbreak in Zanzibar was caused by *Vibrio cholerae* serogroup O1, El Tor biotype and serotype Ogawa that was similar in all three sourced samples- fish, sewage and patients. The O1 El Tor biotype, since the sixth global pandemic, has slowly replaced the Classical O1 biotype and has been responsible for the seventh global pandemic [23]. This biotype is currently prevalent in the East and Central African region and the world [44,24] and it was the major biotype reported by Eibach [6] in Ghana 2011 cholera outbreak when 97% isolates were *ctxB Vibrio cholerae* El Tor biotype. Previous work on *Vibrio cholerae* in Zanzibar has also identified El Tor biotype [45]. Pathogenic potential of the present isolates are marked by possession of O1 antigen and CT production manifested by severe diarrhoea in the outbreak that claimed 48 lives. It is worth noting that case fatality rate (CFR) in Zanzibar (1.8%) was higher than the 1% targeted by WHO [13].

In the 2015/16 cholera outbreak CFR were higher in the above 5 yrs group (62.5%) which is in contrast to what was reported by UNICEF where worldwide the CFR was higher in <5yrs group [9]. Low mortality rates in less than 5 yrs group may be attributed to prudence practiced by mothers who happen to have high attendance for training in infant clinics in Zanzibar [46]. Morbidity was highest in 16-30 (37.6%) age group followed by 6-15 and 31-50 yrs age groups-both 19.4%. The two age groups 16-30 and 31-50 are supposed to be most active and probably their mobility exposes them to infection. Andrews [47] also noted that human mobility played a great

role on spread of *Vibrio cholerae*. Other reason could be consumption of non-hygienic vended foods as noted by Dzotsi et al. [48]. Morbidity was slightly higher in males (52.5%) than females (47.5%) but surprisingly mortality was markedly higher in males (62.5%) than females (37.5%). This could be due either to negligence in males in taking action after first symptoms appear or, males being an active group, are away from home when symptoms are first manifested hence the delay to report for early therapy. Study in Kenya [49] showed that most deaths occur among persons who had not sought early medical treatment.

Antibiotic drug resistance in *Vibrio cholerae* has been reported worldwide [50]. Resistance has been reported against ampicillin, erythromycin, nalidixic acid, tetracycline, streptomycin, kanamycin, trimethoprim, sulphonamides and gentamicin. In this study resistance was observed against Nalidixic acid (93%) which is the first generation fluoroquinolone but, on the contrary none of the isolates was resistant to Ciprofloxacin, the second generation fluoroquinolone. In contrast other studies reported resistance to Ciprofloxacin [16]. While Tran et al. [51] reported all isolates from Vietnam outbreak were resistant to co-trimoxozole this study observed 60% resistance.

Close to half (47%) of isolates were resistant to tetracycline. This is in not in agreement to what was found by Shrestha et al. [1] in Katmandu cholera outbreak when tetracycline was the most effective and drug of choice for cholera treatment. On the other hand Taneja et al. [36] observed even higher resistance (66.7%) of *Vibrio cholerae* isolates to tetracycline compared to what was observed in this study. The reason for wide variations on tetracycline resistance patterns could be due to instability of plasmids in *Vibrio cholerae* that carry tetracycline resistant genes [36]. Moreover class I integron, SXT constin, *tetG* and *tetA* genes have also been reported to be associated with the spread of genetic determinants of resistance to antimicrobial agents including tetracycline [52].

Multidrug resistance (MDR) was observed in 40.0% of the isolates which is higher than the 6.45% resistance reported by Gupta et al. [35] in Nepal outbreak or Tran et al. [51] (7%) in Vietnam outbreak. Shrestha et al. [1] however, reported all isolates in Nepal *Vibrio cholerae* outbreak were multidrug resistant and possessed a *ctx* gene of approximately 400 base pairs. High resistance to tetracycline and nalidixic acid together with high rates of multidrug resistance in this study are causes of concern on drug resistance in cholera therapy in Zanzibar.

Gene sequencing of *ctxB* gene in this study proved a homogeniety of isolates from fish, sewage and clinical cholera patients belonging to the same cluster, hence the possibility of fish as one of the fomites for transmission of cholera in Zanzibar. Moreover previous studies have shown that *Vibrio cholerae* can live symbiotically in fish intestines by breaking down chitinous materials [53]. Apart from fish the *Vibrio cholerae* bacteria can also associate with a variety of zooplankton, phytoplankton, and algae which can act as a media of horizontal gene transfer [18].

Isolates in this study were related with those from Haiti and China which are far distant from East Africa. While transmission from geographically separated areas can't be ruled out in today's mobile world, it is also possible to find *Vibrio cholerae* with similar lineage that evolved in solitary independent ecosystems [51,16,44]. It was also found in this study that the Zanzibar 2013 *Vibrio cholerae* isolates belonged to 3 different clusters that genetically differed from the 2015/16 cluster. This observation can be due introduction of novel strains from outside Zanzibar or presence of rapidly emerging endemic reservoirs of *Vibrio cholerae* in the Zanzibar environment that give rise to distinct genotypes in each outbreak.

**Table 3. Antimicrobial susceptibility pattern of *Vibrio cholerae* isolates towards commonly used antibiotics**

| Antimicrobial | Number of isolates | Sensitive isolates n (%) | Resistance isolates n (%) |
|---|---|---|---|
| Ampicillin | 45 | 24(53) | 21(47) |
| Chloramphenicol | 45 | 39(87) | 6(13) |
| Ciprofloxacin | 45 | 45(100) | 0(0) |
| Co-trimoxazole | 45 | 18(40) | 27(60) |
| Erythromycin | 45 | 15(33) | 30(67) |
| Nalidixic acid | 45 | 3(7) | 42(93) |
| Tetracycline | 45 | 24(53) | 21(47) |

**Table 4. Drug resistance phenotypes of *Vibrio cholerae* isolates**

| Antibiotic resistance pattern | Number of strains |
|---|---|
| CHL,AMP,NAL,TE | 3 |
| AMP,NAL,TE,ERY | 6 |
| NAL,TE,ERY | 3 |
| AMP,NAL,TE | 6 |
| COT,AMP,TE,ERY | 3 |
| COT,NAL,ERY | 12 |
| COT,NAL, | 6 |
| COT,CHL,NAL,ERY | 3 |
| COT,AMP,NAL,ERY | 3 |
| Total number of strains | 45 |

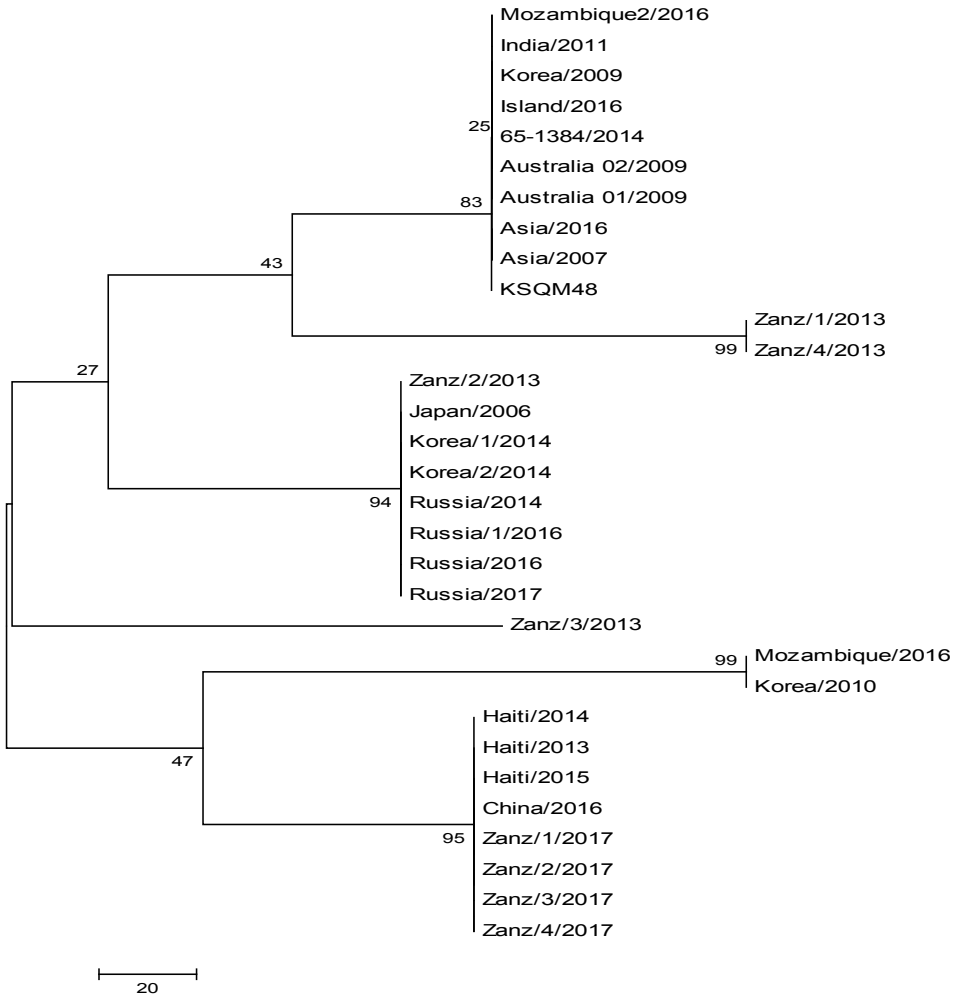


**Fig. 1. Phylogenetic tree of nucleotide sequences of the *ctxB* gene showing the phylogenetic relationship of *Vibrio cholerae* recovered from sewage drains, marine fish and human samples during the 2015/2016 outbreak in Zanzibar, Tanzania. The tree was obtained by the neighbour-joining method calculated with the Jukes and Cantor model. Bootstrap testing of phylogeny was performed with 1000 replications and percentage values are indicated in the branches**

**Table 5. Reference isolates and strains of *Vibrio cholerae* obtained from the gene bank and referred in the Phylogenetic tree**

| Country | Accession number of isolate | Year |
|---|---|---|
| Russia | KM352500 | 2014 |
| Russia | KU215666 | 2016 |
| USA (KSQM 48) | CP006947 | 2015 |
| Russia | KT779273 | 2016 |
| China | CP013307 | 2016 |
| Russia | KX584734 | 2017 |
| India | HQ04514 | 2011 |
| Mozambique | FF158842 | 2016 |
| Zanzibar | KX312666 | 2013 |
| Zanzibar | JX144324 | 2013 |
| Zanzibar | JX312670 | 2013 |
| Australia | EU828583 | 2009 |
| Australia | EU828588 | 2009 |
| Iran (65-1384) | JN132472 | 2014 |
| Iceland | AF452582 | 2016 |
| Korea | GQ485649 | 2010 |
| Korea | KJ540258 | 2014 |
| Haiti | CP007634 | 2014 |
| Korea | KJ540264 | 2014 |
| Haiti | CP003069 | 2013 |
| Korea | FJ449754 | 2009 |

## 4. CONCLUSION

Generally this study proved that fish, either through cross contamination or consumption of poorly cooked fish foods, could play a role on cholera transmission in Zanzibar. *Vibrio cholerae* serogroup O1, biotype El Tor serotype Ogawa was isolated from all three sources fish, sewage and stool of human patients. Homogeneity of isolates was confirmed by genome sequencing of *ctxB* gene. Relatedness of Zanzibar 2017 *Vibrio cholerae* isolate with Haiti and China isolates and its distinction from Zanzibar 2013 isolate bear evidence of genetic versatility of the *Vibrio cholerae* bacteria, its ability to evolve in solitary independent ecosystems and possibility of presence of rapidly e emerging endemic reservoirs of *Vibrio cholerae* in the Zanzibar environment. High antibiotic resistance was observed against five of the seven antibiotics tested and 40% of isolates were multidrug resistant, a finding that clinicians, public health authorities and Ministry of Health need to monitor and act accordingly.

## CONSENT

All authors declare that 'written informed consent was obtained from patients or close relatives before stool samples were collected for publication of this manuscript.

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by Zanzibar medical ethics committee (ZAMREC) for specimen collection from Mnazimmoja hospital and laboratory analysis (Protocol Number: ST/0004/July/016). Approval was also obtained from municipal authorities, Ministry of Agriculture and Fisheries in Zanzibar and State University of Zanzibar (SUZA) for sample collection from sewage drains and fish respectively and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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