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Prevalence, Pathogenic Markers and Antibiotic Susceptibility of *Vibrio cholerae* in Sardines, Water and Phytoplankton in Lake Tanganyika, Tanzania

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Abstract

The aim of this study was to evaluate the extent of *Vibrio cholerae* contamination and their antibiotic resistance patterns in sardines, water and phytoplankton in Lake Tanganyika, Tanzania. A total of 120 samples from sardines, water and phytoplankton were collected and analysed for *V. cholerae*. All isolates confirmed as *V. cholerae* using Polymerase Chain Reaction were also determined for virulence genes and antibiotic susceptibility. Sardine samples (9%) harboured *V. cholerae* (non-O1) and water samples (3%) harboured *V. cholerae* O1. *Vibrio cholerae* was not detected and isolated in phytoplankton samples. One isolate from water samples harboured both toxin regulatory protein (toxR) and haemolysin gene (hlyA), but not cholera enterotoxin gene (ctx) and toxin co-regulated pilus gene (tcpA). *Vibrio cholerae* isolates were resistant to Ampicillin (83.33%), Amoxicillin (100%), Chloramphenicol (50%) and Tetracycline (100%). All of the isolates were susceptible to Gentamicin and Ciprofloxacin. The study demonstrated that, fish and water are important reservoirs of *V. cholerae*. Regardless the absence of ctx and tcpA, constant monitoring for *V. cholerae* should be done as they pose threat to human health.

Keywords

Cholera, Fish, Virulence Genes, Sardines

1. Introduction

The human population surrounding Lake Tanganyika depends on the fish found in the lake [1]. The fishery of Lake Tanganyika is of great importance to the surrounding regions since fish are important source of food, provide employment and increase income. In the lake, there are two major types of commercial fish (sardines); small fish namely *Stolothrisa tanganicae* (locally known as Dagaa) and the bigger ones *Limnothrisa miodon* (locally known as Lumbo) that are native fish species of Lake Tanganyika. Both smaller and bigger fish contribute over 60% to the catches of Lake Tanganyika where Tanzania is a major exporter of fish from the lake as compared to the other countries sharing the lake [2].

Although fish from the lake are good sources of proteins, but they are associated with enteric bacterial contamination like *V. cholerae*, *Salmonella*spp and *E. coli* [3]. Despite, the ability of pathogenic vibrios to cause disease depends on the expression of various virulence-factors like toxin-coregulated pilus [4]. *Vibrio cholerae* is among the major

cause of food-borne infections with a considerable social-economic cost to the affected populations. Every year millions of cholera episodes occur throughout the world especially in developing countries [5]. Tanzania has also been reporting the disease almost every year since the seventh cholera pandemic reached the country in 1974 [6, 7].

However, high prevalence of cholera in Kigoma region is reported at alarming rate since 1978 [8]. Cholera outbreak in human is caused by *V. cholerae* that contaminate water and foodstuffs including fish [9]. Moreover, cholera outbreak is associated with poor sanitation practices and unhygienic environmental condition of the lake. Fish and water serves as vehicles for the transmission of Vibrio species in human, especially if water used for washing sardines is contaminated with *V. cholerae*. Contaminated fish and water increases the incidence of cholera outbreak and hence more burden to human health [10]. The effect associated with cholera to affected individuals include, severe dehydration and death within hours if left untreated. This leads to great economic losses due to loss of manpower and cost in disease control and prevention. There are limited studies that have been conducted to establish the extent of bacterial contaminants in sardines and water in Lake Tanganyika, thus it necessitated the need to investigate the magnitude of *V. cholerae* from environmental samples in Lake Tanganyika. On the other hand, a significant increase in the prevalence of *V. cholerae*'s resistance to antibiotic agents was reported [11]. As result, a test for antibiotic susceptibility was carried out. The findings from this study therefore serve as baseline information and an input to policy makers for developing preventive and control measures of *V. cholerae* in Lake Tanganyika and other lakes in the countrywide.

2. Materials and Methods

This study was carried out from October, 2015 to February, 2016. The study was carried out in Kigoma Municipality in areas along the Lake Tanganyika (Tanzania side). The sites included Kibirizi fish landing site (3-4 km from Kigoma town) and Katonga fish landing site (a small village in Bangwe Division situated 4-5 km to the south of Kigoma Bay). Samples of fresh sardines (about 100 g) were purchased from local fishermen immediately after landing at the beach and then stored overnight at Tanzania Fisheries Research Institute (TAFIRI), Kigoma. Fresh sardines were stored in freezer at -18°C, water and phytoplankton were stored in a fridge at 2-8°C. Samples were transported in cool box with ice cubes at about 2-8°C to National Fish Quality Control Laboratory (NFQCL) Nyegezi, Mwanza for testing. Dry sardines were stored and transported at ambient temperature (20-25°C). About 250 ml of water samples were collected in 250 ml sterile bottles from surface water and stored in sterile cool box with ice cubes (2-8°C). Approximately 100 ml of phytoplankton samples were collected at the depth of about 10 m of the Lake Tanganyika. The Phytoplankton samples were collected according to APHA [12], whereby 100 litres of surface water was filtered through a phytoplankton net of 13µm mesh size. The concentrate that remained at the bottom of the net (about 100 ml) was collected into a sampling bottle for laboratory analysis. The distance of sampling point for water and phytoplankton were 100 m, 200 m and 300 m offshore. Samples were collected from the same area in the Lake in monthly intervals during fishing period for five months. A total of 120 samples including 66 sardines, 30 water and 24 phytoplankton were collected. Out of 66 sardine samples, 33 were from Katonga and 33 from Kibirizi landing sites. Out of 30 water samples, 15 were from Katonga and 15 were from Kibirizi. Out of 24 phytoplankton samples, 12 were from Katonga and 12 from Kibirizi.

2.1. Isolation of *V. cholerae*

The isolation of *V. cholerae* was carried out as per Tanzania Bureau of Standards [13]. Briefly, 25 g of sardines (about 20-25 whole pieces of sardines) samples were homogenised with 225 ml of APW (Oxoid Ltd, Basingstoke, Hampshire, England) to make the first initial suspension. Another 25 g of sardines samples were homogenised with 225 ml of GPSB (Oxoid Ltd, Basingstoke, Hampshire, England) to make the initial suspension. The homogenate suspensions were incubated at 37°C for 24 hours for enrichment. One hundred ml of water samples were concentrated on 0.45 µm pore diameter membrane filter (Millipore, Bedford, USA) and enriched in APW. Similarly, phytoplankton were concentrated on 0.45 µm pore diameter membrane filter paper (Millipore, Bedford, USA) and transferred into APW. Concentrated water and phytoplankton samples were incubated at 37°C for 24 hours. A loop full of enriched culture samples from APW was streaked on TCBS agar plate (Oxoid Ltd, Basingstoke, Hampshire, England) and those GPSB enrichment culture were streaked on GPA plate (Oxoid Ltd, Basingstoke, Hampshire, England). All the plates were incubated at 37°C for 24 hours. After incubation, yellow and shinning colonies on TCBS agar and red colonies from GPA plates were suspected as *V. cholerae* and purified on Saline Triple Sugar Iron Agar (STSI) plate (Oxoid Ltd, Basingstoke, Hampshire, England). Purified colonies were screened by Gram staining, samples that were Gram negative and commas shaped were tested for Oxidase reaction (BDH Chemical LTD, England). Positive samples (colour changed to blue or dark purple within 10 seconds) were tested by STSI slant for species confirmation. Uniform yellow colour colonies with no production of Hydrogen sulphide gas (H_2S) after overnight incubation at 37°C were regarded as presumptive *V. cholerae*. Thereafter, sero-agglutination test was performed using specific *V. cholerae* O1 anti-serum.

2.2. Molecular Identification of Toxigenic *V. cholerae*

The DNA was extracted using a commercial kit - QIAamp DNA blood Mini Kit (QIAGEN GmbH, Hilden, Germany). The extraction of DNA was done according to the manufacturer's instructions. Briefly, total DNA was purified

from cultured *V. cholerae*; the sample was mixed with 180 µl of lysis buffer and 20 µl proteinase in a 1.5 ml microcentrifuge tube, then mixed by vortex and incubated in the heat block at 56°C for 1 hour. After incubation, the mixture was briefly centrifuged to remove drops from inside of the lid of the 1.5 ml microcentrifuge tube, then mixed with 200 µl buffer for 15 seconds and incubated at 70°C for 10 minutes. 200 µl ethanol (96 – 100%) was added to the sample and centrifuged, the spin column was washed with two buffers (500 µl buffer AW1 and 500 µl AW2) followed by addition of Elution Buffer that extracted DNA taped on the column membrane. The assay was conducted by conventional PCR amplification using GeneAmp PCR System 9700 PCR machine (Applied Biosystems, Foster City, CA, USA). Specific genes namely; the outer membrane protein (ompW), cholera toxin (ctx), toxin co-regulated pilus (tcpA), toxin regulator (toxR) and haemolysin (hlyA) were targeted as shown in table 1. For gel electrophoresis, 10 µl of PCR products was loaded into a horizontal 1.5% agarose gel stained with 0.1 µl/ml of DNA marker GelRed (Phenix Research) dived in 1xTBE (Tris Borate EDTA) buffer. Electrophoretic separation was performed at 100 V for 1 hour along with 1000 (bp) PCR ladder as molecular weight marker. The gel was visualized under UV trans-illuminator and recorded using digital camera. Double distilled DNase free water was used as negative control and DNA from reference strain of *V. cholerae* O139 NCTC 12945 (ATCC 51394) (Salisbury, SP4 OJG, UK) was used as positive control.

Table 1. *Primers sequences used for the PCR.*

Targeted genes	Primer Sequences (5'-3')	Size (bp)	Source
Toxin regulator (*toxR*)	F-CGG GAT CCA TGT TCG GAT TAG GAC AC R-CGG GAT CCT ACT CAC ACA CTT TGA TGG C	900	[14]
Outer membrane protein *(ompW*)	F-CACCAAGAAGGTGACTTTATTGTG R-GAACTTATAACCACCCGCG	588	[4]
hlyA (Haemolysin)	F-GGC AAA CAG CGA AAC AAA TAC C R-CTC AGC GGG CTA ATA CGG TTT A	727	[15]

2.3. Antibiotic Susceptibility Testing

All confirmed positive isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method [16]. The following antibiotic discs namely Tetracycline (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Ampicillin (10µg) and Amoxicillin (10µg) (Oxoid Ltd, Basingstoke, Hampshire, England) were used. The inhibition zone diameters were measured using a transparent plastic ruler and interpreted according to the zone diameter interpretive chart of CLSI [17].

2.4. Data Analysis

Proportions of positive *V. cholerae* samples at different sites were calculated and then compared by Chi-square and Fisher exact tests according to the total sizes using EPI-INFO 7 statistical software. Statistical significance was defined at a probability of $p = 0.05$.

3. Results and Discussion

3.1. Prevalence of *V. cholerae* in Sardines, Water and Phytoplankton

Vibrio cholerae from the isolates were identified by detecting Outer membrane protein (ompW) using primers with the expected 588bp. Out of the sixty six sardines samples, only six (9%) were positive for *V. cholerae*. Samples contaminated with *V. cholerae* from Katonga landing site were high (15%, n=33) compared to Kibirizi landing site (3%, n=33) samples. However, the difference was insignificant at $P>0.05$. The prevalence of *V. cholerae* in water was (3%) out of 30 samples, however the prevalence was confirmed to be *V. cholerae* O1. The isolate was from Kibirizi landing site (6%, n= 15) samples and none of *V. cholerae* from Katonga landing site (0%, n= 15) samples was isolated. The difference was not significant at 95% level of confidence ($p>0.05$). Prevalence of *V. cholerae* found in fish and water from this study is comparable to previous studies in Israel and Burkina Faso [10, 18]. The study found 71% and 6% prevalence of *V. cholerae* from fish and water respectively. Likewise in Tanzania *V. cholerae* were isolated from fish and water and reported prevalence of 53.7% in surface, 17.1% in gills, 4.9% in intestine and 20% in water [19]. Although, *V. cholerae* isolated from sardines in this study were not of the serotypes causing cholera, they may still cause sporadic cases of watery diarrhoea and inflammatory enterocolitis. The type of *V. cholerae* isolated in this study from water is the main cause of human diarrhoea. Being water borne infection, cholera is transmitted by ingesting food or water contaminated with the bacterium [20]. Several studies have isolated the organism from fish and various water sources and associated them with hospitalised patients with diarrhoea, peritonitis and also in immunocompromised cases [4, 21, 22]. In researches conducted in Bangladesh and Thailand demonstrated that, *V. cholerae* was the important cause of diarrhoeal disease in humans [21, 22].

Twenty four phytoplankton samples were collected from two different landing sites and none of the *V. cholerae* was isolated from these samples. This may be due to failure of bacterium to adopt a viable state in phytoplankton when the environmental conditions change, such as low concentrations of nutrients and temperatures. Viable state enables them to carry out metabolic functions and form colonies without

being culturable. However, they remain present in the aquatic environment throughout the year, either in free-living or in association with phytoplankton [23].

3.2. Characterization of *V. cholerae*

After identification of *V. cholerae*from sardines and water, all isolates were subjected to PCR for detection of virulence genes. The toxin regulator (toxR) was identified in one isolate (*V. cholerae* O1 serogroup) from water sample. The gene was identified by PCR using specific primers at 900 bp (Figure 1). This finding is similar to what was reported in Kenya, the 24% of toxR gene in *V. cholerae* O1 from environmental strains were isolated in the coastal and Lake Victoria Basin regions [24]. This gene controls the coordinated expression of genes associated with pathogenicity in toxigenic *V. cholerae*. The haemolysin (hlyA) gene was identified by PCR using specific primers shown by a 727 bp sized amplicon (Figure 2), one isolate (*V. cholerae* O1 serogroup) from water sample was identified. Study conducted in Tanzania showed the presence of hlyA gene in one of the *V. cholerae* O1 isolated from fish collected from stabilisation ponds [25]. The gene is very important for bacterium; it confers to the *V. cholerae* cells with an ability to cause blood cell lysis in the infected host. All *V. cholerae* isolates from sardines and water were further tested for the presence of cholera enterotoxin gene (ctx) and cholera toxin co-regulated pilus subunit A (tcpA) by PCR using specific primers at 167 bp and 453 bp respectively. It was found that, neither O1 nor non-O1 *V. cholerae* isolates contained ctx and tcpA operon. Genes were amplified on the positive control *V. cholerae* only. These findings correspond with the study conducted in India and reported the absence of tcpA and ctx in *V. cholerae*non-O1 [14]. Further studies in Burkina Faso reported that, non-O1 *V. cholerae* in fish and water lacked the ctx gene [18]. Although organisms of the O1 serogroup are frequently isolated from aquatic environments, most of the environmental *V. cholerae* O1 isolated do not produce cholera toxin to which the clinical state of cholera is principally attributed [26].

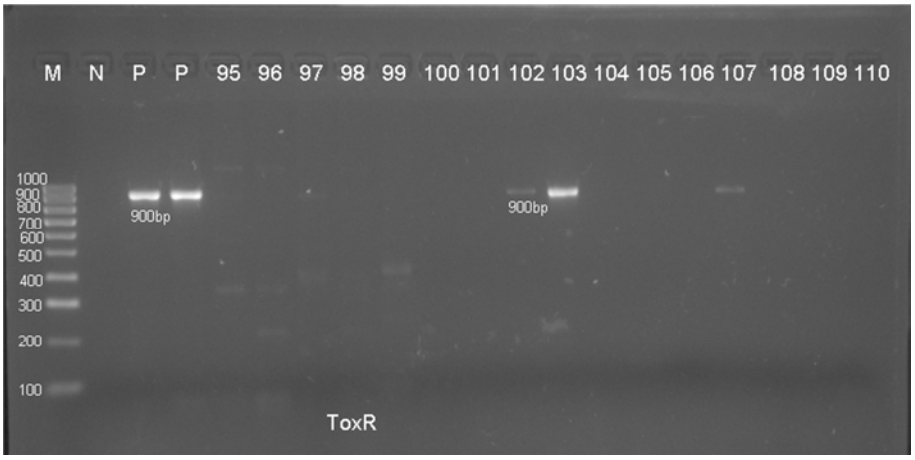

Figure 1. *Cholera toxin regulatory protein detected in one isolate (Sample No 103) using PCR.*

M: DNA ladder; lanes 95 to 110 are *V. cholerae* DNA samples; N: Negative Control (DNA free water); P: Positive control (VC 0139, ATCC 51394)

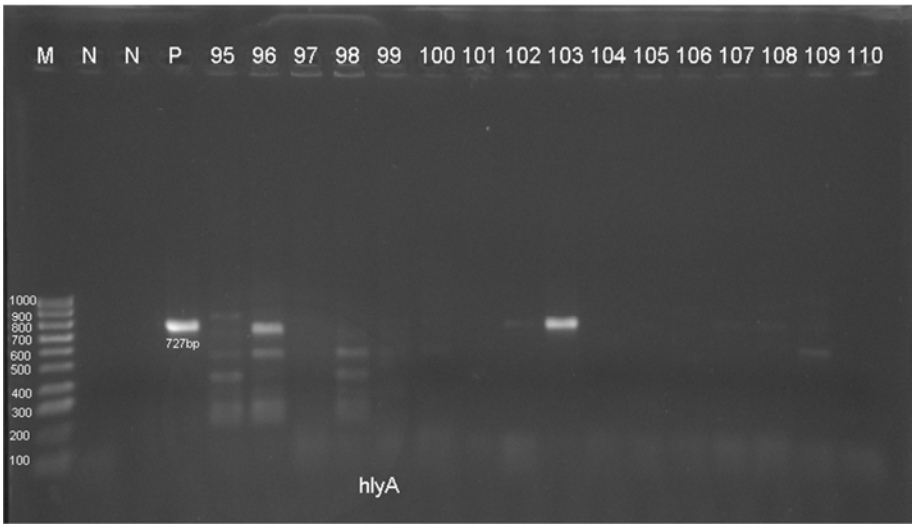

Figure 2. *Haemolysin gene (hlyA) detected in one isolate (Sample No 103) using PCR.*

M: DNA ladder; lane 95 to 110 are *V. cholerae* DNA samples; N: Negative Control; P: Positive control

3.3. Antibiotic Susceptibility Patterns

The antibiotic susceptibility testing was carried out to the isolated *V. Cholerae*strains. *Vibrio cholerae* isolates showed resistance to Chloramphenicol (50%), Ampicillin (83.33%), Tetracycline (100%) and Amoxicillin (100%). Findings of this study accentuate the study conducted in New Bell-Douala, Cameroon that showed Ampicillin resistance (92%), Amoxicillin (88%) and Tetracycline (68%) [11]. Likewise, the study conducted in Kenya showed that; *V. cholerae* O1 isolates from water and fish samples in Lake Victoria Basin of western Kenya were resistant to Tetracycline and Ampicillin (66.7%) [27]. On the other hand *V. cholerae* O1 strains isolated in a tertiary-care centre in India were resistance to Ampicillin (64.3%) [28]. The study conducted in Burkina Faso showed that, *V. cholerae* isolates from fish and water were resistant to Ampicillin (50%) [18]. Although findings of this study show 50% resistance to chloramphenicol, the study conducted in Cameroon showed that 80% were susceptible to chloramphenicol [11]. Resistance to the mentioned antibiotics may be related to their misuse in humans and veterinary medicine [29, 30, 27, 31]. However, Tetracycline is one of the antibiotics mostly used in Kigoma region and as well in country wide for cholera treatment [32]; this may therefore, play a role in persistence and dissemination of pathogenic strains in study area. In this study, *V. cholerae* isolates were sensitive to Ciprofloxacin (100%), and Gentamicin (100%). The antibiotic sensitivity in these antibiotics is comparable to the previous findings conducted in Uzebba and Tanzania showed the susceptibility of Ciprofloxacin in *V. cholerae* [18, 32, 25]. Ciprofloxacin has been used in Kigoma region for treatment in humans during cholera outbreak [33], the findings thus agree with its use in cholera treatment. WHO also recommends the use of Ciprofloxacin as treatment choice for cholera [34].

4. Conclusion

Humans are infected by *V. cholerae* due to ingestion of contaminated water and food including fish. *Vibrio cholerae* were isolated from sardines and water in Lake Tanganyika. The PCR results for detection of virulence genes revealed that, the *V. cholerae* O1 isolate was harbouring two pathogenic markers; toxR and hlyA genes. Despite the fact that *V. cholerae* isolated in this study had no ctx and tcpA, the presence of *V. cholerae* in the study area signifies potential of cholera-like diarrhoea and extra intestinal infections in humans. It should also be noted that in the samples where the *V. cholerae* was not detected, environmental conditions could be unfavorable for its growth (viable but non-culturable form-VNC). Under favorable climate conditions VNC *V. cholerae* could revert to transmissible state, cholera control strategies in this endemic area should be encouraged even when *V. cholerae* is not detected in some samples. Despite the *V. cholerae*isolates displayed increased resistance towards Chloramphenicol, Ampicillin, Amoxicillin and Tetracycline; still they were susceptible to ciprofloxacin and Gentamicin. *Vibrio cholerae* are the causative agents of cholera epidemics and endemics; therefore, identification and detection of *V. cholerae* is very important for providing epidemiologic and public health information.

Conflict of Interest

Authors of this publication had no conflict of interests.

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References

[1] United Republic of Tanzania 1998. Kigoma Region Socio-Economic Profile: ThePlanning Commission Dar es salaam and Regional Commissioner's Office Kigoma, Tanzania.

[2] Kirema-Mukasa, C. T. 2012. *Regional Fish Trade in Eastern and Southern Africa-Products and Markets*: A Fish Traders Guide. Smart Fish working papers No. 013.

[3] Sichewo, P. R., Gono, R. K., Muzvondiwa, J. V. and Sizanobuhle, N. 2013. Isolation and Identification of Pathogenic Bacteria in Edible Fish: A Case Study of Fletcher Dam in Gweru, Zimbabwe. *International Journal of Science and Research* (IJSR) 9 (2): 269-273.

[4] Sathiyamurthy, K., Athmanathan, B. and Subbaraj D. K. 2013. Prevalence of *Vibrio cholerae*and Other Vibrios from Environmental and Seafood Sources, Tamil Nadu, India. *British Microbiology Research Journal* 3 (4): 538-549.

[5] Ali, M., Nelson, A. R., Lopez, A. L. and Sack, D. A. 2015. Updated Global Burden of Cholera in Endemic Countries. *PLoS Neglected Tropical Diseases* 9 (6).

[6] World Health Organization 2008. Cholera Country Profile: United Republic of Tanzania. [http://www.who.int/cholera/countries/TanzaniaCountry-Profile2008.pdf]. Accessed on 12 August 2016.

[7] World Health Organization. Cholera: situation in WHO African region as of 03 June 2013. [http://www.afro.who.int/en/clusters-a-programmes/dpc/epidemic-a-pandemic-alert-and-response/3852-cholera-situation-in-the-who-african-region-as-of-03-june-2013.html]. Accessed on 17 October 2016.

[8] Rutagemwa, D. K. 2010. Establishment and Adoption of a Water Quality Monitoring System, Introduction of the Lake Water Monitoring Programme, and Sharing Information widely in the Lake Tanganyika Basin Draft Report.

[9] Lutz, C., Erken, M., Noorian, P., Sun, S. and McDougald, D. 2013. Environmental reservoirs and mechanisms of persistence of *Vibrio cholerae. Front Microbiol*ogy 4: 375-380.

[10] Senderovich, Y., Izhaki, I. and Halpern, M. 2010. Fish as reservoirs and vectors of *Vibrio cholerae*. *PLoS ONE* 5 (1): 1-5.

[11] Akoachere, J. F. T. K., Masalla, T. N. and Njom, H. A. 2013. Multi-drug resistanttoxigenic *Vibrio cholerae* O1 is persistent in water sources in New Bell-Douala, Cameroon. *BMC Infectious Diseases* 13: 366.

[12] American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Waste Water 20th Edition. United Book Press Inc., Baltimore, Maryland. pp 2-16.

[13] Tanzania Standards 2002. Examination for *Vibrio cholerae* in food stuffs. TZS 733.

[14] Kondo, S. and Ajawatanawong, P. 2009. Distribution and sequence analysis of virulence associated genes in *Vibrio cholerae* O1, O139 and non-O1/non-O139 isolates from Thailand. S*outheast Asian Journal of Tropical Medicine Public Health* 40 (5): 1015-1024.

[15] Fooladi, I. A. A., Islamieh, I. D., Doust, H. R., Karami, A. and Marashi, S. M. 2013. Design of a multiplex PCR method for detection of toxigenic pathogenic in Vibrio cholerae. Asian Pacific Journal of Tropical Medicine 2013: 115-118.

[16] Nhung, P. H., Ohkusu, K., Miyasaka, J., Sun, X. S. and Ezaki, T. 2007. Rapid and specific identification of 5 human pathogenic Vibrio species by multiplex polymerase chain reaction targeted to dnaJ gene. *Diagnostic Microbiology and Infectious Disease* 59: 271–275.

[17] Clinical and Laboratory Standards Institute 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. *Wayne, PA* 34 (1).

[18] Traoré, M., Martikainen, O., Siitonen, A., Traoré, A. S., Barro, N. and Haukka, K. 2014. Occurrence of *Vibrio cholerae* in fish and water from a reservoir and a neighbouring channel in Ouagadougou, Burkina Faso. *Journal of Infection in Developing Countries* 8 (10): 1334-1338.

[19] Mdegela, R. H., Mhongole, O. J., Kamundia, P. W., Byarugaba, D. and Mbuthia, P. G., 2015. Identification of *Salmonela* and Vibrio in water and *Oreochromisniloticus* in Mwanza Gulf, Lake Victoria, Tanzania. *International Journal of Current Research* 7 (7): 18087-18092.

[20] World Health Organization 2011. Cholera Fact Sheet Number 107.[http://www.who.int/mediacentre/factsheets/fs107/en/index.html]. Accessed on 16 July 2015.

[21] Albert, M. J., Siddique, A. K., Islam, M. S., Faruque, A. S. G., Ansaruzzaman, M., Faruque, S. M. and Sack, R. B. 1993. A large outbreak of clinical cholera due to *Vibrio cholerae* non-O1 in Bangladesh. *The Lancet* 341 (8846): 704.

[22] Dalsgaard, A., Forslund, A., Bodhidatta, L., Serichantalergs, O., Pitarangsi, C., Pang, L., Shimada, T. and Echeverria P. 1999. A high proportion of *Vibrio cholerae* strains isolated from children with diarrhoea in Bangkok, Thailand. Multiple antibiotic resistant and belong to heterogenous non-O1, non-O139 O-serotypes. Department of Veterinary Microbiology. The Royal Veterinary and Agricultural University, Denmark.

[23] Huq, A., Haley, B. J., Taviani, E., Chen, A., Hasan, N. A. and Colwell, R. R. 2012. Detection, Isolation, and Identification of *Vibrio cholerae* from the Environment. Current Protocols in Microbiology.

[24] Kimani, R. W., Muigai, A. W. T., Sang, W., Kiiru., J. N. and Kariuki, S. 2014. Virulence factors in environmental and clinical *Vibrio cholerae* from endemic areas in Kenya. *African Journal of Laboratory Medicine* 3 (1) 7 pp. [http://dx.doi.org/10.4102/ajlm.v3i1.41] site visited on 5th July 2016.

[25] Hounmanou, Y. M. G., Mdegela, R. H., Dougnon, T. V., Mhongole, O. J., Mayila, E. S., Malakalinga, J., Makingi, G. and Dalsgaard, A. 2016. Toxigenic *Vibrio cholerae* O1 in vegetables and fish raised in wastewater irrigated fields and stabilization ponds during a non‑cholera outbreak period in Morogoro, Tanzania: An environmental health study. *BMC Research Notes* 9: 466.[https://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-016-2283-0] Site visited on 21st October, 2016.

[26] Igbinosa, E. O. and Okoh, A. I. 2008. Emerging Vibrio species: An unending threat to public health in developing countries. *Research in Microbiology* 159: 495-506.

[27] Onyuka, J. H. O., Kakai, R., Onyango, D. M., Arama, P. F., Gichuki, J. and Ofulla A. V. O. 2011. Prevalence and antimicrobial susceptibility patterns of enteric bacteria isolated from water and fish in Lake Victoria basin of western Kenya. *World Academy of Science, Engineering and Technology* 5: 3-22.

[28] Mandal, J., Dinoop, K. P. and Parija, S. C. 2012. Increasing antimicrobial resistance of *Vibrio cholerae* O1 biotype El Tor strains isolated in a tertiary-care centre in India. *Journal of Health, Population and Nutrition* 30 (1): 12-16.

[29] Kagashea, G. A. B., Minzia, O. and Matoweb, L. 2010. An assessment of dispensing practices in private pharmacies in Dar-es-Salaam, Tanzania. *Interanational Journal of Pharmacy Practice* 19 (1): 30-35.

[30] Tagoe, D. and Attah, C. 2010. A Study of Antibiotic Use and Abuse in Ghana: a case study of the Cape Coast Metropolis. *The Internet Journal of Health* 11 (2).

[31] Katakweba, A. A. S., Mtambo, M. M. A., Olsen, J. E. and Muhairwa, A. P. 2012. Awareness of human health risks associated with the use of antimicrobials among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. Livestock for Rural Research Development 24 Suppl 10 Article no. 170.

[32] Ukaji, D. C., Kemajou, T. S., Ajugwo, A. O., Ezeiruaku, F. C., Eze, E. M. 2015. Antibiotic Susceptibility Patterns of *Vibrio cholerae* O1 isolated during cholera outbreak in Uzebba. *Journal of Bioscience and Bioengineering* 2 (3): 33-36.

[33] Centers for Disease Control and Prevention (2015). Department of Health and Human Services, Public Health Service. Potential cholera exposure in Kigoma, Tanzania.

[34] Sack, D. A, Lyke, C., Mclaughlin, C., Suwanvanichkij, V. 2001. *Antimicrobial Resistance in Shigellosis, Cholera and Campylobacteriosis*. World Health, 8-21.