# LABORATORY SURVEILLANCE OF CHOLERA IN NYANZA PROVINCE DURING THE OUTBREAK FROM APRIL TO JULY 2007

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**Abstract**
Cholera continues to be an important cause of morbidity and mortality in many areas of the world, and there is currently a high frequency of new outbreaks in Africa. Following a confirmed cholera outbreak in Siaya, Kisumu, Bondo and Nyando districts, Nyanza province in western Kenya between April and July 2007, a laboratory surveillance study was conducted at the New Nyanza Provincial General Hospital's Microbiology Laboratory. The study aimed at isolating and identifying the strain of *Vibrio cholerae*, Identifying the mean age of the patients and monitoring the susceptibility patterns to major antibiotics. It further aimed at determining effectiveness of empiric management of cholera. A total of 219 samples were processed out of which a total of 85 samples (39%) were found positive for *Vibrio cholerae* 01 sero-type Inaba. The mean age recorded was 19 years (1 ≤ 80). The modal ages recorded were 8, 20 and 25. 55% (47) of the recorded cases were females while 45% (38) were males. Generally, *V.Cholerae* 01 sero-type Inaba showed antibiotic resistance to trimethoprim-sulfamethoxazole, nalidixic acid, sulfasoxazole, streptomycin and furazolidone. Tetracycline a commonly used antibiotic for empiric management was 100% effective on all isolates and remains the drug of choice. Samples obtained for case-control study did not yield any cholera isolate. No prior exposure to any antibiotic was recorded among all the subjects. The study confirmed the effectiveness of empirical therapy on cholera and further identified the need of proper hygiene, water treatment, proper waste management and proper eating habits as means of controlling morbidity and mortality of cholera.

**Key words**: Cholera, morbidity, mortality, empiric management, antibiotic resistance

## 1.0 Introduction

Cholera continues to be a global threat leading to significantly high morbidity mortality (Salim *et al*., 2005). From 1971, Kenya has continually suffered several waves of cholera outbreaks with the largest epidemic to date reported in 1997 affecting 17,200 (Shapiro *et al*., 1999). Between 1997 and 1999, the final official figures covering the three years put Kenyan epidemic at 10% of all cholera cases reported from the African continent during that period (WHO, 2000).

Cholera outbreaks are linked to crowded living conditions, inadequate or unprotected water supply and poor sanitation, conditions which are rampant in many developing countries, making almost every developing country vulnerable to cholera outbreaks (WHO, 2003). The risk of cholera epidemics is intensified during man-made and natural disasters, such as conflicts and floods, and when large populations are displaced. Explosive outbreaks with large numbers of deaths are often the results of such cholera attacks (WHO, 2003).

The outbreaks, further cause panic, leaving affected countries to deal with a double burden of economic consequences such as those that are the direct result of travel and economic sanctions imposed by other countries as well as the cost of managing the epidemic. For example, following cholera outbreak in East Africa, affecting millions of people in more than 10 countries in 1997, trade sanctions were imposed on fish exports from these East African countries (WHO, 1997).

From September, 2006, Kenya experienced various cholera outbreaks in different parts of the country. Between September and October the same year, twenty four and eight cases of cholera with one and three death cases respectively were reported in Mombasa and Kwale districts respectively (GOK, 2006). Just after cholera cases were reported in the neighboring country of Somalia in January 2007, Kenya experienced sporadic outbreaks in the neighboring Wajir and Mandera districts. During the same time outbreaks were also reported in West Pokot, Kisumu, Siaya, Bondo and Nyando Districts.

Currently, in Kenya response to cholera is often reactive and takes the form of an emergency response, often with inadequate preparedness. Although these responses on many occasions prevent many deaths, they fail to prevent cholera cases on a long-term basis. This study embarks on adding baseline data that will facilitate cholera management. It aimed at isolating and identifying the strain of *Vibrio cholerae*, identifying the mean age of the patients, monitoring the susceptibility patterns to major antibiotics and determining effectiveness of empiric management of cholera.

## 2.0 Materials and Methods

### 2.1 Study Sites

The study took place during the cholera outbreak in parts of Nyanza province between April and July 2007, covering mainly Kisumu, Bondo, Nyando and Siaya districts. Nyanza province is located in western Kenya and the Districts are mainly inhabited by a rural community bordering Lake Victoria, mainly populated by the Luo ethnic group. Economic activities here are fishing, cattle keeping and subsistence farming. Rainfall is seasonal, and occurs generally from March to May and from October to December. Apart from Kisumu town, other areas of the region are characterized by poor water system, where borehole, pond, river and water from the lake are used for domestic purposes.

Nyanza is one of Kenya's most impoverished provinces. As at 1997, 63 % of the population lived below poverty line (Shori R, 2000). This number has increased in the recent past. The province has one of the nation's lowest immunization rates, highest infant mortality and highest prevalence of HIV that is 22 % among adults 15 - 49 years old in 2000 ( Min of Health and NASCOP 2001). Malnutrition is also common while endemic diseases include malaria, tuberculosis and schistosomiasis.

### 2.2 Surveillance and Specimen Collection

Following the outbreak of cholera in Kisumu District, spilling over to the neighboring Districts of Siaya, Bondo and Nyando districts within one week span in April/May 2007, a laboratory- based surveillance was conducted at the New Nyanza Provincial Hospital's Microbiology Laboratory. Following the spill over, a second diagnosis site was established at Siaya District Hospital Laboratory. Samples were collected from the sites and transported in Cary-Blair, a transport medium, to the two laboratories in cool containers and processed within six hours. Preliminary results of bacterial cultures were sent to the sites within twenty four hours- these results later tallied with the final results. Sero-grouping, sero-typing and antimicrobial susceptibility test were performed and the results once more sent to clinic staff at the sites and the Provincial Health Management Team, to guide therapy. Samples were also sent to the KEMRI/CDC microbiology laboratory in Kisian for External Quality Control, the results which tallied with those obtained in the laboratory.

### 2.3 Laboratory Procedures

Upon arrival at the New Nyanza Provincial General Hospital's microbiology laboratory, processing was done in a two way blind fold method where any specimen was subjected to both *Vibrio* and non-*Vibrio* but pathogenic bacteria. Samples were cultured in TCBS (Thio-sulfate Citrate Bile Salt), XLD, Selenite F and Alkaline peptone water. Biochemical tests were done using TSI or KIA, LIA and motility test media. Other preliminary investigations included oxidase tests, string tests and Gram staining. Typing was done using PV 01, INABA, OGAWA and 0139 antisera. Non *Vibrio* cases were identified using *Shig* "O" or polyvalent "H" and PV

"O" for *Shigella* and *Salmonella* respectively. Isolation and identification were done using convectional bacteriological methods by Farmer *et al* (1991).

### 2.4 Antibiotic Susceptibility Test

After positive serological identification, antibiotic susceptibility test was done using Kirby-Bauer disk diffusion method on the Mueller Hinton Agar (Cheesbrough 2002) and interpretations done using NCCLS guidelines (NCCLS 1997). A total of 12 selected antibiotic disks including chloramphenicol (C), Trimethoprim-sulfa (STX), tetracycline (TE), Ciprofloxacin (CIP), Nalidixic acid (NA), Ampicillin (AM), Sulfisoxazole (G), Streptomycin (S), Kanamycin, Gentamycin (GM), Ceftriaxone (CRO) and Furazolidone (FX) were used. General trend of antibiotic testing took into account pregnant/lactating mothers and the presence of children. Discs were chosen to accommodate both parental and oral antibiotic testing. Results were recorded as Susceptible (S), Moderately Susceptible (MS), Intermediate (I) and Resistant (R). Organism for control used was *Escherichia coli* ATCC 25922. This was a laboratory surveillance study during an outbreak. All samples were tested once on arrival at the laboratory. Isolates that were moderately Susceptible (MS) or Intermediate (I) were repeated a second time. If conflicting results were obtained, the susceptibility tests were repeated a third time.

### 2.5 Case-Control Study

During this same period, a total of thirty randomly sampled stool specimens for persons attending New Nyanza Provincial General Hospital's out patient department and Chulaimbo Provincial Rural Health Center were selected for analysis. These patients presented stomach complaints with or without diarrhea. Excluded from the study included those who had traveled outside their localities five days before the onset of the complaint and those coming from the cholera affected regions.

### 2.6 Statistical Methods

The data obtained were entered into spreadsheets for statistical analysis. The mean ages and percentage prevalence were calculated using ms-excel. Other analysis were done using SPSS version 10. For continuous variables, means were compared by student's t-test and $p$ values $\leq 0.05$ were considered significant.

## 3.0 Results

### 3.1 Surveillance

Between April 28$^{th}$ and July 2007, a total of 299 cases of cholera were recorded from Siaya, Kisumu, Nyando and Bondo districts, Nyanza province in Western Kenya. Out of these recorded cases, a total of 219 samples were processed both at Siaya District Hospital Laboratories and at the New Nyanza Provincial General Hospital's Microbiology Laboratory.

Of the processed (219) samples, a total of 85 samples (39.7%) were found positive for *Vibrio cholerae* sero-group 01 sero-type Inaba, while 2 samples (2.4 %) were found positive for *Vibrio parahemolyticus*. The mean age recorded was 19 years with the minimum and the maximum ages recorded being 1 year and 80 years respectively. The modal ages recorded were 8, 20 and 25 while ages were not recorded for 4 subjects. 55 % (47) of the recorded cases were females while 45 % (38) were males. *V.cholerae* sero-type Ogawa or sero-group 0139 was not isolated. By the end of this study, Siaya had recorded 139 cases with 59 samples confirmed positive for *Vibrio cholerae* sero-type *inaba*, and 9 death cases. Bondo recorded 6 cases with 1 death case, whereas Nyando reported 24 cases with 2 death cases (Table 1). During the surveillance period in Nyanza province, the last cases were recorded at the New Nyanza Provincial Hospital in July, 2007.

*Table 1: Samples received and processed at the New Nyanza Provincial General Hospital and Siaya District Hospital Laboratories April to July 2007*

| **Study site** | **Cases Recorded** | **Samples Processed** | **Positive Samples for *V.cholerae* 01 inaba** |
|---|---|---|---|
| **(District)** | **(n= 299)** | **(n= 219)** | **(n= 85)** |
| Kisumu | 130 | 50 | 17 |
| Siaya | 139 | 139 | 59 |
| Nyando | 24 | 24 | 8 |
| Bondo | 6 | 6 | 1 |

*Vibrio cholerae* sero-type Inaba was found more frequently in young school going children and young adults, (0 - 9) and (20 - 29), accounting for 34 % and 26 % respectively (Figure 1), while minimal occurrence was observed among those between 50 - 59 years. These results reflected the initial cases reported during the outbreak, which mainly came from four primary schools in Kisumu West district of the province. The mean age of infection in the case study was 25 years as opposed to 19 years realized in the cholera surveillance study. Only 1 (14 %) case was less than 9 years in the case control with the highest case 3 (43 %) being between 30 and 39 years as opposed to 50 to 59 and 0 to 9 years being the least and the highest age brackets respectively recorded in the cholera surveillance study

The antimicrobial susceptibility tests were only done on 31 out of the total 85 isolates. Generally, *V.cholerae* sero-type *Inaba* showed resistance to trimethoprim-sulfamethoxazole, nalidixic acid, sulfasoxazole, streptomyscin and furazolidone. Susceptibility to chloramphenicol, ampicillin and kanamycin were recorded as 74 %, 87 % and 97 % respectively, while Moderate susceptibility results were recorded for chloramphenicol (19 %) and kanamycin (3 %). Intermediate range was recorded for Chloramphenicol (3 %). Tetracycline, a commonly prescribed drug for cholera, was found to be 100 % effective. Antibiotic susceptibility tests were

done for these cholera isolates with Ofloxacin (Ofl) and amoxillin- clavulanic (Amox-Clav) acid replacing streptomycin and furazolidone respectively. This study reports complete resistance by *Vibrio cholerae* to Trimethoprim-Sulfamethoxazole, Nalidixic acid, Sulfisoxazole and Furazolidone (Table 2).

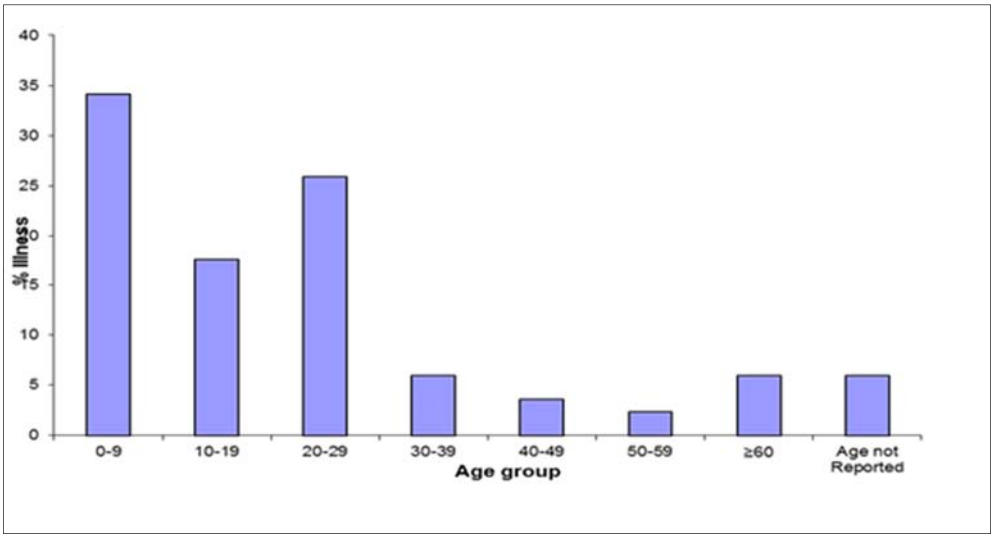


*Figure 1: Cholera cases by age (n=85) in Nyanza province, May to July 2007*

*Table 2: Antibiotic susceptibility for 31 out of 85 cholera positive isolates isolated during the cholera outbreak in Nyanza province, April to July 2007*

| Site | Samples Tested (n=31) | Susceptile Isolates CHL | STX | TE | CIP | NA | AMP | G | S | K | GM | CRO | FX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Kisumu** | 17 | S 82% (14)<br>Ms 18% (3) | 0 | 100% (17) | 100% (17) | 0 | S 82% (14)<br>Ms18% (3) | 0 | 0 | 100% (17) | 100% (17) | 100% (17) | 0 |
| **Siaya** | 5 | S 40% (2)<br>Ms 20% (1)<br>I 20% (1) | 0 | 100% (5) | 100% (5) | 0 | 100% (5) | 0 | 0 | 100% (5) | 100% (5) | 100% (5) | 0 |
| **Nyando** | 8 | S 75% (6)<br>Ms25% (2) | 0 | 100% (8) | 100% (8) | 0 | S 88% (7)<br>Ms 12% (1) | 0 | 0 | S 88% (7)<br>Ms12% (1) | 100% (8) | 100% (8) | 0 |
| **Bondo** | 1 | 100 % (1) | 0 | 100% (1) | 100% (1) | 0 | 100% (1) | 0 | 0 | 100% | 100% (1) | 100% (8) | 0 |

CHL: Chloramphenicol; STX: trimethoprim-Sulfamethoxazole; TE: Tetracycline; CIP: Ciprofloxacin; NA: Nalidixic Acid; AMP: Ampicillin; G: Sulfisoxazole; S: Streptomycin; K: Kanamycin; GM: Gentamycin; CRO: Ceftriaxone; FX: Furazolidone .**S:**Susceptible **MS:**Moderately Susceptible **I:** Intermediate **R**: Resistant.

In the case-control study, complete resistance to trimethoprim-sulfamethoxazole was once more observed on all the isolates. All isolates in the case control showed resistance to tetracycline. Susceptibility patterns to other drugs varied among different isolates. *Salmonella* spp showed complete resistance to Trimethoprim-Sulfamethoxazole and Tetracycline with varied susceptibility patterns to other antibiotics Likewise Salmonella typhi recorded resistance to the two antibiotics, but with additional resistance to Ampicillin. However, the bacteria only showed moderate susceptibility to Sulamethoxazole, with intermediate susceptibilities to other commonly used antibiotics. In addition to Trimethoprim – Sulfamethoxazole and Tetracycline, Shigella spp also recorded further to Ampicillin.

A brief assessment of Siaya District which had a relatively higher incidence revealed that majority of the cases did not have adequate latrines for different household, this number contributing to 46.7 % (21) of the total with cases recording having no latrines at all in the homes accounting for 37.8 % (17) (Table 3). Sources of water varied among the cases, however majority of the patients appeared to have been using stream water from "Fludhi" stream. One case recorded was a student from a local primary boarding school and therefore it could not be reported with certainty the source of water or use of latrine or toilet.

*Table 3: Domestic water sources and latrine use by cholera cases from Siaya District during cholera outbreak in Nyanza province April to July 2007 (n=45)*

| **Water source** | | **Presence of Latrine** | | | |
|---|---|---|---|---|---|
| | **Number of samples** | **Proper latrine use in the home** | **Inadequate for household use** | **No latrine** | **Other** |
| Borehole | 7 | 1 | 3 | 3 | |
| Protected well | 1 | | 1 | | |
| Rivers | | | | | |
| Nzoia | 3 | 1 | 2 | | |
| Futuro | 3 | | | 3 | |
| Not specified | 4 | | 4 | | |
| Roof Catchment | 1 | 1 | | | |
| Streams | | | | | |
| Fludhi | 15 | 2 | 5 | 8 | |
| Gaoya | 1 | | 1 | | |
| Nyahulunyu | 1 | | | 1 | |
| Wuoroya | 1 | | 1 | | |
| Not specified | 2 | 1 | 1 | | |
| Piped water | 2 | | 2 | | |
| Water source not reported | 4 | 1 | | 2 | 1 in Pr. Boarding school |
| **TOTAL** | **45 (100 %)** | **6 (13.5 %)** | **21 (46.7 %)** | **17 (37.8 %)** | **1 (2 %)** |

## 4.0 Discussion and Conclusion

This study established the cause of cholera outbreak during this period in Kisumu, Bondo, Siaya and Nyando districts during the period of April to July 2007 to be *Vibrio cholerae* sero-group 01 sero-type Inaba. The study further reports susceptibility to tetracycline, a drug commonly used in empiric management of cholera. Susceptibility to tetracycline is a clear indication that there were no antibiotic resistant mutant cholera strains circulating in the region during this outbreak. Therefore empiric management of cholera in this region is still feasible. The disease had highest prevalence in school going children of 0-9 years and in young adults 20-29 years. High prevalence of bacterial diarrhea among children from 1997 to 2003 was also reported by Brooks *et al.,* (2003) in the same region. However, this high prevalence in children would be expected, due to the fact that elevated risk in young children is partly explained in the increased faecal-oral contamination and decreased immunity (De Las Cases *et al*., 1999).

Infection with HIV may have been an important co-morbidity for diarrheal diseases including cholera infection among young adults in this community, where an estimated 22% of young adults are infected (Ministry of Health and NASCOP 2001). However, during the study HIV status of the subjects was not ascertained and therefore these findings cannot ascertain the burden of cholera and its etiologies among HIV infected and un-infected persons. An alternative explanation for high prevalence realized in this study, among young adults, is that young people with greater appetite ingest more bacteria and thereby increase their risks to infection.

The high prevalence realized in young children, and due to the fact that house hold chores are mainly undertaken by women, may partly explain the fact that more females than males were infected. Women mainly take care of children and therefore increase their risk of contracting infectious diseases. Further, the fact that the first cases were occured among school going children from different schools may point towards a common activity bringing together the schools. In such events women are known to sell cooked food to these children. There are therefore chances that food contaminated with *Vibrio cholerae* could have been sold to the first cases recorded. This study does not however report with certainty this fact as primary case tracing was not within the scope of this study.

Antibiotic resistance in enteric pathogens is of great importance in developing world where the rate of diarrheal diseases is highest. Thus, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern (Periska *et al*., 2003). Bacteria are becoming more resistant to most potent antibiotics. The overuse of certain antibiotics may contribute to the development of resistant strains. The study realized resistance to furazolidone among *Vibrio cholerae* isolates tested in this study. However, this was not a new

finding as resistance to furazolidone has previously been reported (Snyder and Merson 1982; WHO, 2001).

Awareness of substantial geographical variations in the common causes of diarrhea has led some groups to recommend regional susceptibility patterns and the choice of empirical management. This study did not establish any results to justify the same as all sites gave similar antibiotic resistance patterns in all the four study sites. The same trend was also seen in the West Pokot outbreak (GOK, unpublished data, 2006). Resistance to some common antibiotics observed during the study also reflected findings of a similar study during the cholera outbreak in India in the year 2000 (WHO, 2000). It was also found out that empiric management of cholera during outbreaks is still effective as tetracycline; a commonly prescribed antibiotic for cholera during the outbreaks was 100 % effective in the entire samples tested.

The 1997 cholera outbreak implicated drinking water from Lake Victoria to the spread (Shapiro et al., 1999). However this study did not find any indicator to the same as some patients in the control study mainly used water from the lake for domestic purposes. An assessment of cases from Siaya district who were the majority revealed that majority of the cases either had inadequate latrines (46.7 %) or no latrines at all (37.8 %). This could be a pointer to poor hygienic standards among this population. Earlier on in the study it was realized that majority of the patients who had proper latrines, though complained of gastroenteritis, no *Vibrio cholerae* or any other bacterial pathogen could be isolated from their stools with a few only recording the presence of Ova and Cysts or puss cells. Likewise, majority in this assessment in Siaya reported using borehole or stream water, specifically from "Fludhi" stream. However the results obtained could not link the stream to the source, as majority again who used this stream did not have any latrines in the homes. Therefore our study suggests that lack of proper hygiene was the main contributer to cholera morbidity among this population.

The findings here therefore, highlighted several opportunities for controlling cholera morbidity. Proper hygiene, use of treated water, use of toilets or latrines and proper eating habits were found to be appropriate. Majority in the control study were from the urban center or areas with these attributes. Apart from failure to asses the HIV status of the subjects, the study had other limitations. Mobile food vendors (hawkers) could not be traced and their samples were not taken for analysis. Investigation of the case control did not take into account previous exposure to prophylactic measures against cholera infections. These are also important factors in cholera study.

We conclude that the April to July 2007 study on the cholera outbreak in Nyanza province identified *V.cholerae* Ser-group 01, Sero-type *Inaba* as the causative agent of the disease. The highest prevalence rate was found in school going children and young adults with more females being affected than males. There were no new

antibiotic resistance patterns observed among the commonly prescribed antibiotics, therefore no mutant strains of cholera were isolated during the study. Finally the study confirmed that empiric management of cholera in the region was effective as tetracycline still remains the drug of choice for empiric management.

When cholera occurs in an unprepared community, deaths among those affected can reach 50 %. In contrast, a well organized and adequate response in an affected country can limit this death rate to less than 1 % (WHO, 2003). Therefore control of cholera should encompass: Improved surveillance to obtain better data for risk assessment and the early detection of outbreaks; Improved preparedness to provide a rapid response to outbreaks and limit their spread; Improved case management to reduce deaths among cases; Improved environmental management to enhance prevention; Accelerated research on the burden of cholera and how best to manage the growing problem of drug resistance. Use of available vaccines according to evidence based guidelines; Partnerships with politicians, the media and the community and finally health education focused on behavioral change.

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