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COMBATING CHOLERA EPIDEMICS BY TARGETING RESERVOIRS OF INFECTION AND TRANSMISSION ROUTES: A REVIEW
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H. D. N. NYAMOGOBA, A. A. OBALA and R. KAKAI

ABSTRACT

Objectives: **To determine the parameters which can be investigated for prevention and effective control of cholera.**
Data sources: **Literature search on compact disk-read only memory (CD-ROM), medline and internet, using the key words: cholera outbreaks, and cholera transmission. A few reviews were manually reviewed.**
Study selection: **Relevant studies or articles on cholera outbreaks and transmission worldwide, with special reference to Kenya is included in the review.**
Data extraction: **From individual study or articles.**
Data synthesis: **Information on cholera epidemics worldwide and in Kenya is synchronized under the headings; Introduction, History and predisposing factors, Current situation, Bioecology and transmission patterns, and, Use of molecular epidemiological and geographic information system (GIS) techniques in mapping out the bioecology, reservoirs and transmission routes of cholera.**
Conclusion: **Cholera can be prevented and controlled more effectively at environment level. This requires a multi-disciplinary approach including poverty alleviation.**

INTRODUCTION

Cholera is a waterborne disease(1) and the El Tor and Classical biotypes of *Vibrio cholerae* are associated with human disease, which may be endemic with sporadic cases or epidemic/pandemic(2,3). It is essentially a disease of poverty and lack of sanitation, preferentially affecting the poor, both persons and nations. Most epidemics are characterised by high morbidity and mortality particularly in crowded urban slums and rural areas where health facilities and clean water are in short supply.

HISTORY AND PREDISPOSING FACTORS

Cholera has been a dreaded disease from the very early times, periodically ravaging the world in the form of epidemics/pandemics. It is now confined to developing and underdeveloped countries(3,4). Indo-China had for centuries been the "home of cholera", hence the name "Asiatic cholera". The disease had largely been confined to the two countries up to the second decade of the twentieth century(3). Between 1817 and 1961, cholera spread by sea routes causing six world pandemics and then retreated to its Asian home(4). The outbreaks in developing countries are particularly associated with poverty and poor sanitation(1).

The El Tor vibrios, originally isolated from the El Tor quarantine station at Sinai peninsula in Egypt(3), were responsible for the seventh pandemic that began in 1961 in Indonesia and by 1971 had ravaged other Asian countries and some parts of Europe. Parts of South America such as Peru and Colombia were also affected. The eighth pandemic however, started to invade sub-Saharan Africa around 1970, and it had spread to South America in the early 1990's. A new and more virulent strain, *V. cholerae* strain 0139, is now spreading westwards from South Asia and is on its way to Europe(5). Similar epidemics swept through Eastern Africa countries spreading fast after the two-month (1997) disastrous El Nino flooding in many parts of the region. Hundreds of lives were lost, and by December 1997, the death toll stood at 650 in Kenya, 248 in Somalia, 1700 in Tanzania, 122 in Zanzibar and 41 in Djibouti.

The predisposing factors for cholera include poor sanitation, poverty and some socio-cultural practices. In the less developed nations, slums and rural areas dwellers often lack potable water, proper sanitation and adequate health services. Some cultural practices tend to aggravate the situation by facilitating the spread of the disease, for instance, eating at funerals where hygiene standards can hardly be maintained due to water and utensil scarcity. Bathing and swimming along rivers also expose community members to infection. In spite of the serious cholera outbreaks, no concrete sanitation steps, for example, building of toilets and boiling drinking water, have been put in place by the rural and urban poor to minimise cholera outbreaks. Admittedly, lack of clean water is a

persistent problem among rural poor and slum dwellers in Kenya. This has resulted in outbreaks of faecal-oral waterborne diseases such as cholera, bacillary and amoebic dysentery and typhoid. According to Migori District Development Plan for 1997 to 2001, there are very few springs and rivers in the district, and the few water points available are polluted and can be real health hazards. Another problem that might lead to cholera outbreaks is the shortage of sanitary toilets. In Migori District for instance, only about 40% of families have pit latrines(6). Even the few toilets that exist are not fully utilised due to taboos which discourage sharing of this facility among family members and relatives, for example father-in-law versus daughter-in-law, thus creating serious sanitary problems.

Cholera outbreaks in Kenya claim many lives because of unpreparedness and inadequate health services. For instance, during the 1997-1998 epidemics which occurred as a result of El Nino floods, more than 650 people died of the disease. The Lake Victoria Basin (LVB), where the disease is endemic, carries the heaviest burden. This is due to the frequent sporadic and occasional epidemics of cholera outbreaks as a result of floods during rainy seasons, scarcity of portable water and poor sanitation.

CURRENT SITUATION OF CHOLERA

The current cholera outbreaks have indicated changing epidemiology of the disease, with the outbreaks occurring in increased frequency even in regions it had never occurred previously(7). Some of these outbreaks are caused by newly emerging strains(8-10). Outbreaks lasting up to one year have occurred in Nigeria and India with fatality rates exceeding 5%(1,11). A study by Kaur *et al*(1) in India showed yearly isolation rates of 2%, 2.6%, 6.7%, 7.08%, 0.9% and 2.6% for the years 1992-1997, respectively. Until 1992, *V. cholerae* 01 Ogawa was the predominant strain, while in 1993, 81.3% of the isolates were 0139 Bengal. However, from 1994 through to 1997, 01 Ogawa was the dominant strain with no 0139 Bengal strain isolated.

In Bangladesh, however, *V. cholerae* 0139 Bengal emerged in 1992 and rapidly spread in an epidemic form replacing the existing 01 strains(12,13). Mukhopadhyay *et al*(14) have also reported strains of *V. cholerae* 0139 to be currently prevailing in India. Gupta *et al*(15) have isolated 64 (13.47%) *V. cholerae* El Tor and Ogawa biotypes from 475 samples processed during the 1994 cholera outbreak in an arid region in India that had never been affected by the disease before. The subsequent emergence of a new clone of *V. cholerae* 01 El Tor biotype transiently displaced the 0139 vibrios during the 1994 epidemic in Bangladesh and the recent re-emergence of the *V. cholerae* 0139 and its co-existence with the El Tor vibrios demonstrates temporal changes in cholera epidemiology(9,13). The rapid changing epidemiology of cholera has therefore become a public health challenge(11,16,17) requiring global monitoring and re-assessment of monitoring techniques.

In Kenya, the latest epidemic started in June, 1997 and by December the same year, the Ministry of Health (MOH)(18) reported more than 555 deaths. In the same period, more than 17,200 people were reported to have contracted the disease in Nyanza, Coast and Nairobi provinces. By January 1998, the death toll stood at 256 in Coast Province alone, with Mombasa District incurring the highest number of deaths (153 deaths), followed by Kilifi 32, Tana River 21, Malindi 20, Kwale 18, and Taita Taveta 12. In the same period (1997 - 1998), more than 15,000 cases of diarrhoea and related diseases had been reported in the province. Similarly, in the city of Nairobi more than 250 cholera-related cases were reported over the same period. The congested slums of Korogocho, Kibera and Mathare were affected by this filth related ailment, with over 30 deaths reported between November and December 1997.

Nyanza province was the most affected, with the epidemic ravaging many districts particularly Migori which was the first to report the epidemic. Later, epidemics were also reported in Rachuonyo, Suba, Homa-Bay and Muhoroni area in Nyando District. The epidemic claimed more than 300 lives in the province. Cholera related deaths were also reported elsewhere in Kenya such as Busia District in Western Province where 23 cases were reported, Machakos in Eastern Province reported 12, while Uasin Gishu and Trans Nzoia Districts in Rift Valley Province reported 10 and three deaths, respectively.

Another serious consequence of the frequent cholera outbreaks in Kenya is the negation of the government's efforts to alleviate poverty. The direct discharges of wastewaters into Lake Victoria and cholera outbreaks have interfered with the sale of fish both locally and internationally, adversely affecting the economy of the lake region. For instance, the recent banning of fish exports from Lake Victoria waters by the European Union markets deprived Kenya of a crucial source of foreign exchange. In addition to this, the communities who depend on fish have been deprived of a source of income and as a result there is increased poverty in the lake region.

CHOLERA BIOECOLOGY AND TRANSMISSION PATTERNS

The most important aspects of the cholera outbreaks in Kenya and elsewhere are the seasonality of epidemics and the ecological niches where the vibrios inhabit and survive in between the epidemics. These aspects of the bioecology and epidemiology of cholera require explanations, which will guide public health authorities in instituting control measures against the disease.

Cholera vibrios have been recovered from water wells, water mains, rivers, sea- water and marine crustaceans such as crabs and Crayfish(19,20). Organic matter and trace salts of up to 2% in water are believed to be necessary for the vibrios' survival in water, but not longer than a week(20). However, it was the continued survival outside the human host, of the El Tor biotype

which had led to local sporadic outbreaks for many years prior to its re-emergence in Indonesia in 1961. In Japan, non - El Tor vibrios were also found in an estuary into which a ditch from a hospital waste tank drained. The vibrios seem to have been breeding in the tank used for disposal of artificial kidney dialysate(21).

Currently an ecosystem- based view of cholera transmission indicates that the spread may be often associated with perturbations of natural ecosystems. Evidence is accumulating that marine phytoplankton such as algae provide refuge for the dormant vibrios(22,23). Prolonged survival of vibrios has also been associated with diatoms, drifting dinoflagellates, seaweed macroalgae, water hyacinth and zooplankton(24,25). When adverse conditions like shift in temperature, pH, salinity and nutrient levels occur, the vibrios contract and hibernate, only to re-emerge in infectious states when the waters warm-up again(25).

The spread of cholera also seems to be influenced fundamentally by levels of eutrophication. For instance, the fluctuations of the disease in Bangladesh have been associated with coastal algae blooms, and hibernating forms discovered using DNA probes(7). These recent findings suggest a possible new pathway for cholera transmission and human-induced and natural disturbances of coastal and other water ecosystems play an important role in the transmission(21).

Speculation is rife in Kenya that cholera radiates from the Lake Victoria region. The presence of hyacinth in the lake and algal blooms found in many water bodies in the region are indicators of water pollution. The vibrios and other enteric pathogens such as *Salmonella, Shigella* and *Entamoeba* may take advantage of this and survive longer in such ecological niches. There is speculation that the water body inclusions may provide suitable conditions which support prolonged survival of the cholera vibrios out of the human body. These could be the points from where epidemics radiate.

The vehicles of cholera transmission vary markedly from place to place, being influenced by local customs and practices. Traditionally water was recognised as the primary vehicle for cholera transmission. However, in the past 30 years outbreaks have been associated with ingestion of contaminated food, although in many instances water is the source of contamination(26,27). Seafood is commonly associated with cholera since it may be contaminated in its natural environment or during preparation. Other food items also associated with outbreaks include fresh vegetables, fruits, meat and poorly cooked grains. Vegetables are usually contaminated with sewage in soil and fruits when washed or injected with contaminated water to increase weight and turgor. Food handling by infected persons who do not observe proper hygiene is also a source of contamination. Refrigeration, freezing, alkaline pH, high concentration of carbohydrate, humidity and absence of competing flora enhance the survival of cholera vibrios in contaminated food(26).

Similarly, faecal contamination of water sources(11,16), late presentation for treatment and low levels of knowledge about cholera infection favour the spread of the disease in communities(16). Densely populated areas and poorly planned cities are most affected. More cases of cholera also seem to occur during rainy seasons, but fatality rates do not show these seasonal variations(11).

USE OF MOLECULAR EPIDEMIOLOGICAL AND GEOGRAPHIC INFORMATION SYSTEM (GIS) IN MAPPING OUT THE BIOECOLOGY, RESERVOIRS AND TRANSMISSION ROUTES OF CHOLERA

The molecular characterisation of cholera vibrios is currently being increasingly used in cholera epidemiology(17). Determination of evolutionary relationships and molecular diversity of the vibrio biotypes is necessary in order to understand sources, origin, and epidemiology of cholera. Amplified restriction fragment length polymorphism (ARFLP) analysis has been used by Jiang *et al*(10) for this purpose. The study used two sets of restriction enzyme - primer to establish the genetic diversity of clinical and environmental 01, 0139, non-01 and non-0139 isolates of V. *cholerae* serogroups. Amplification of Hind III - and Taq I-digested genomic DNA produced 30-50 bands for each strain and was able to separate environmental isolates of 01 and non-01 strains, while amplified fragment length polymorphism analysis of restriction enzymes Apa 1- and Taq-1 digested genomic DNA yielded 20-30 bands for each strain and was able to separate 01 from 0139 strains.

Analysis of the restriction fragment length polymorphisms (RFLPs) in genes for the conserved rRNA (ribotype) by Faruque *et al*(9) in Bangladesh also revealed the recently isolated *V. cholerae* 0139 strains to belong to a new ribotype which is distinct from the previous ribotypes of toxigenic V. *cholerae* 0139. In Japan however, Dalsgaard *et al*(12) have ribotyped *V. cholerae* non-01 and non-0139 strains using the restriction enzyme Bgl II. The 103 epidemiologically unrelated strains, mainly clinical, that were typed representing 10 serotypes (02,05,06,07,011, 013,017,024,041, and 0141) yielded 67 different patterns. Ten of the 160141 strains studied contained cholera toxin (CT) gene (ctx) including seven strains recovered from stool and water samples from USA. Comparing ribotype similarity with each serotype using Dice coefficient (Sd), however, showed a low degree of correlation, but both methods appear to be valuable in studying the epidemiology of emerging *V. cholerae* serotypes.

In Japan, Iwanaga *et al*(28) have used RFLP analysis to exonerate imported *V. cholerae* 01 El Tor they isolated from imported fish as the cause of sporadic cases reported in July, 1994. They restricted the DNA of the imported fish and clinical isolates by the Not I enzyme, which produced different DNA fragments. This indicated that there was no relation between the imported and clinical isolates. Mukhopadhyay *et al*(14) have also established that the 0139 strains isolated in the 1996 epidemic in India were

involved in the 1992 epidemic after obtaining two identical restriction patterns by ribotyping the two isolates with Bgl II restriction enzyme. The emergence of the *V. cholerae* 0139 in 1996 after a 32-month quiescent period re-establishes the 0139 serogroup as an entity which is likely to play a crucial role in the temporal antigenic variations among cholera causing V. *cholerae* serogroups.

A recent cholera outbreak in Colombia was investigated by Tomayo *et al*(29), and 173 *V. cholerae* 01 isolates were analysed for cholera toxin gene (ctxA). All the isolates were ctxA positive, which has significant public health implications. Ribotyping with Bgl I restriction endonuclease digestion of total DNA revealed 3 ribotypes; B5a, 165 isolates (96.4%,) B20, 6 isolates (3.5%) and B21a, 2 isolates (1.1%). The B20 and B21a were new isolates. Studies on clonal diversity among V. *cholerae* 0139 by Faruque *et al* (14) suggest that the 0139 Bengal strains with cholera toxin gene (ctx) possibly emerged from the El Tor strain, while the ctx negative non-Bengal 0139 strain might have emerged from a non-01, non-0139 strain, meaning that strains belonging to the 0139 serogroup may have emerged from similar serotype-specific genetic changes in more than one progenitor strain of *V. cholerae*. The emergence of toxigenic *V. cholerae* 0139 (8,10) has changed the scenario of cholera and has as a result led to the development of 0139 phage typing scheme which differs from the old 01 phage typing in their lytic patterns. The new 0139 phage typing scheme comprises of five newly isolated phages which would be another useful tool in the study of *V. cholerae* 0139 epidemiology (8).

Cholera toxin (CT), which is responsible for profuse diarrhoea(30,31) is encoded by lysogenic bacteriophage designated CTXPhi. Molecular epidemiological surveillance has also revealed clonal diversity among lysogenised (toxigenic) V. *cholerae* strains and continual emergence of new epidemic clones. However, lysogenic conversion is a possible mechanism of origination of new toxigenic clones, and their selective enrichment during cholera outbreaks constitute an essential component of the natural ecosystem for the revolution of epidemic *V. cholerae* strains and genetic elements that mediate the transfer of virulence genes. The ecosystem comprising of *V. cholerae*, CTXPhi, the aquatic environment and the mammalian host offers an understanding of the complex relationship between the pathogenesis and natural selection of the vibrios(30).

Changes in the phenotypic and genotypic properties of cholera vibrios require a close monitoring for epidemiological purposes(16,17). In Bangladesh, Faruque *et al*(9) have shown *V. cholerae* 0139 strains to be undergoing considerable re-assortment in genetic elements encoding antimicrobial resistance. In India, Kaur *et al* (1) have also isolated V. *cholerae* 01 Ogawa strains, which were initially sensitive to most antibiotics except cotrimoxazole, but after three years they were isolating strains all of which were 100% sensitive to cotrimoxazole. Initially, the isolates were sensitive to chloramphenicol but a year into their studies they were isolating resistant strains. Radu *et al*(17) are of the opinion that identical antibiotic resistance patterns indicates that the tested isolates share a common mode of developing resistance and multiple antibiotic resistance indexing suggests that the strains tested originate from high risk contamination.

The genetic relatedness of DNA from random amplified polymorphic DNA (RAPD) analysis, and similarity of plasmid profiles of the clinical and environmental isolates show a correlation with the source of infection. Hofer *et al*(32) have shown multiple resistance in cholera vibrios to be encoded by a plasmid transferable by conjugation to *Escherichia coli* K12, with a frequency between $5x10^{-6}$ and $8x10^{-2}$.

Many health problems and quality of life in general are related to environmental quality. In disease epidemiology, the community structure, the pattern of life, the migratory habits and interactions with the environment combine to form a strong database comprising of both geo-referenced and attributable information. When analysed, this often leads to the elucidation of disease determinants and their point source. For instance, past cholera and tuberculosis epidemics in western Europe had been shown to be direct consequences of the poor hygienic circumstances in respect of water supply and the indoor environment(33). However, most information related to environment and health have important spatial component which can be used to explain the spatiality and patterns of health outcomes and environmental phenomena. GIS which is mainly a computer-based software has the means of integrating spatial information and attribute data, and can provide output in an easily understandable form. This is because it has the capacity to show the spatial and temporal distribution of health needs of a community and can generate presentation formats that can be used to address them(34). In spatial epidemiology, disease information may be presented in two formats, firstly, where the available disease data are aggregated to a set of spatial units, and secondly, that the point location of each case is recorded and may be presented as a dot on a map. These types of data are important in locating high-risk areas, as well as mobility pattern of the population in an epidemic condition.

GIS has been used in various parts of the world to assess environmental risks associated with diseases. This demonstrates how GIS could be applied to show up risk factors that contribute to cholera distribution. When GIS is used, it is possible to display environmental risks, cholera incidences and distribution on a map by overlaying attribute data on referral points to show the spatial pattern of its distribution.

The spatial distribution of a disease in a given locality is temporal and spatial as a result of which there will be high concentration of the disease incidence around the causative agent(35,36). Cholera, salmonellosis and shigellosis transmission are most likely to be intense at the fish landing bays and eating points and as a result, it is possible to locate these points on the map after which focused remedial measures can be taken. Elsewhere, Eng *et al*,(37) have used computer-generated dot maps to

examine the spatial distribution of 194 *Toxoplasma gondii* infections associated with an outbreak in British Columbia, Canada and found the incidence of the T. *gondii* among the patients served by one water distribution system to be 3.52 times that among patients served by other sources. Therefore, GIS can greatly assist in establishing the epidemiological response mechanism for detection and location of high risk areas for cholera and other water- and food-borne infections outbreaks such as salmonellosis, shigellosis and amoebiasis. This can be achieved by developing disease database.

CONCLUSION

The epidemiology of cholera is changing rapidly. This has made the disease a public health challenge requiring global monitoring and re-assessment of monitoring techniques. Cholera is more of an environmental than a clinical problem, which should be combated more effectively at the environment level. Waiting for outbreaks to constitute temporary measures such as treating cases would likely increase the operational costs beyond what most developing countries can afford. Cholera outbreaks in developing countries are particularly associated with poverty and poor sanitation. In Kenya for example, the Lake Victoria basin bears the greatest burden of cholera outbreaks as a result of frequent sporadic cases and seasonal epidemics associated with poverty and low hygienic standards. Polluted water bodies provide the ecological niches that prolong the survival of cholera vibrios and it is from these niches that transmission is thought to radiate.

RECOMMENDATIONS

Cholera epidemics may be combated more effectively after establishing the communities' knowledge and beliefs about the cause or source of cholera, and the socio-cultural practices that influence the endemicity, distribution, outbreaks and transmission of the disease. The changes in the phenotypic and genotypic properties of the cholera vibrios require close monitoring. Molecular characterisation of the cholera vibrios into serovars, RFLP types and ribotypes is important for the development of suitable serological diagnostic kits, vaccine(s), and may greatly assist in tracing ultimate sources of infection or contamination in case of outbreaks.

The determination of the bioecology of cholera vibrios and identification of the environmental factors that influence their distribution and survival are necessary for effective control and prevention of outbreaks. In Kenya for example, there is need to map out the cholera reservoirs and transmission routes country-wide starting from the Lake Victoria basin. This would enable public health authorities to institute appropriate measures to control the disease before it becomes endemic throughout the country.

Development of cholera database and establishing epidemiological response mechanisms for detection and location of high-risk areas for outbreaks using geographic information system (GIS) is important for combating cholera epidemics. Locating and mapping out the distribution of reservoirs of cholera vibrios and identifying transmission routes using GIS is important for laying effective strategies for prevention and control of cholera outbreaks. Combating cholera outbreaks requires a multi-disciplinary approach. Health education on personal hygiene, and poverty alleviation may be essential components in the strategies and efforts to prevent and control cholera epidemics.

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