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Research Article

# Molecular and Genomic Epidemiology of Non-O1/Non-O139 and O1/O139 Vibrio cholerae: A Molecular typo-genomics strategy for studying Emerging Potential Environmental Bacterial Pathogens

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**Abstract:** Recent happening of epidemic, sporadic and pandemic associated with bacteria pathogens around the globe continue to arouse fear amongst the populace. Specific examples amongst such pathogens are the non-O1/non-O139 and O1/O139 *Vibrio cholerae* which are implicated in acute watery diarrhea (AWD) and cholera cases. It was reported that more than ten Southern/Eastern African countries have suffered the scourge since the beginning of 2018. Considering the highlights on the rapid transmission or spread of AWD/Cholera and the relative impact on public/environmental health, molecular epidemiology of transmitting agents has shown promising relevance in diverse field of interest. This study reviewed researches on some published articles on Cholera/AWD within the Southern African Development Communities (SADC), other East and West African countries and elsewhere. The role of molecular epidemiological tools in understanding the dynamics of Non-O1/Non-O139 and O1/O139 *Vibrio cholerae* transmission/spread, source tracking of outbreaks and possible management/control in probable and confirmed cases of vibriosis was discussed. It also emphasized on the current updated nature/burden of the pathogen in endemic, epidemic and pandemic area in Southern African countries as the problem of safe-water and hygienic-food for-all continues to resurface. The need for application of molecular and gene based epidemiological approach in profiling bacterial strains cannot be overemphasize especially amongst emerging or reemerging strains of Non-O1/Non-O139 *Vibrio cholarae* and O1/O139 *Vibrio cholerae*.

**Keywords:** Molecular epidemiology, acute watery diarrhea (AWD) and cholera, Vibriosis, Source tracking, genomic and genotyping, SADC.

## How to Cite:

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## INTRODUCTION

According to numerous investigators, the annual burden of cholera cases has been estimated in a range of 1.3 million to 4.0 million with annual death reported cases ranging from 21,000 to 143,000 worldwide (WHO facts, 2018; Ali et al., 2015). The recent pathotype (the seventh pandemic ElTor strains 7PETs) was said to have entered Africa through West African countries (Sierra Leone, Nigeria, Ghana, Liberia and Guinea) via land, rivers, trade routes and international travel. It later spread via similar routes to South Sudan, DR Congo and other African countries were it remains an ongoing cause of death (WHO-UNICEF, 2018; Thompson et al., 2011; Gaffga et al., 2007). Current reports of the African regional data by (WHO-UNICEF 2018) shows that amongst twenty-one Southern and Eastern African countries more than 10 (47.62%), have reported greater than 15,042 cases of AWD/cholera since the beginning of 2018. These recorded cases have also resulted the death of more than 237 infected persons (with case fatality of 1.6%) from the reported cases. The surveillance report of the year (2018) indicates the occurrence of new case of the pathogen in Zambia, Somalia, Malawi, Zimbabwe etc with case fatality rates ranging from 1% - 5%. The endemic nature of cholera was previously reported by (Fillion and Mileno, 2015) in more than 50 countries both in Asia and Africa. In neighboring continents, majority of the reported cases between 2010 and 2012 affected the Western Hemisphere (Haiti) which spread towards the Mexico, Dominican Republic and Cuba with traveling individual as the vehicle for transmission and spread of such infection or cholera. There have also been reports on the distribution/spread of cholera being associated with natural disaster (Jahan., 2016; Soyapi., 2017; WHO., 2018; Igere et al., 2020a,b), while affected areas continues to source for unhygienic/unsafe domestic water and food. Southern and Eastern Africa countries have shown vulnerability to a variety of such sudden/slow-onset of natural disaster, which includes drought, floods, disease epidemics, food insecurity, political restiveness, energy insecurity and many others which have affected millions of people in preceding years (Holloway et al., 2013; Igere et al., 2022a,b,c). These natural occurrences have also influenced spread of diseases in relation to population growth, urbanization, international migration, climate change, water scarcity and degradation of the environmental (Jahan., 2016). The surge has welcomed various interests due to the endemicity, pandemicity, and distribution of the epidemic both in the SADC region, Africa, Asia and other countries in the world. The situation has also informed interests in determining the epidemic of existing pathogens, phenotypic characteristics of the existing types, antimicrobial profiles, plasmid profiles as well as the phenotypic and genetic dynamics of new strains. The former would only be possible when stringent determination protocols are employed including basic molecular biological methods. This is the basis for drive towards the molecular epidemiology of pathogens especially the Non-O1/Non-O139 and O1/O139 *V. cholerae* members that are ravaging the Africa continent.

Molecular epidemiology entails a genotypic determination of targeted virulent pathogens with specific interest on their genomic as well as plasmid DNA composition with an interest in pathogen speciation, strain typing, patho-genomics and DNA sequencing of specific pathogens. It also includes extra-chromosomal DNA profile and phenobolomics of the specified pathogen (Igere et al., 2021a,b; Vilela et al., 2018; Paramithiotis and Drosinos, 2018; Zheng et al., 2017; Xie et al., 2017; El Ghany et al., 2014; Young et al., 2012; Devoto et al., 2011; Struelens, 1996). It involves determining the genetic basis of pathogen and disease caused, including variants strains in hosts, source track pathogens that are infectious, their transmission, and prevention strategies (Igere et al., 2022a,b; Prosperi et al., 2013; Smura et al., 2013; Pérez-Losada et al., 2013; Carey-Ann and Carroll, 2013; Harrison et al., 2013). According to (Eybpoosh et al. 2017), molecular epidemiology is seen as related aspect of epidemiology that employs molecular biology methods/protocols to determine the

distribution, spread/transmission of diseases. It involves using molecular biology techniques to unravel/identify infectious pathogens, mode of transmission, mechanism of spread or circulation pattern, probability of transmission, order of transmission, transmitting vectors or agents' sources/origin or pathogen and reservoirs. It is subdivided into various determinative branches amongst highly virulent pathogens especially the V. cholerae members. These include the molecular epidemiology of polymorphic genetic virulence factors, polymorphic surface lipopolysaccharides (LPS) subunits and polymorphic antibiotic resistant markers. The efficient description of these division would aid deciphering transmission pattern, response to antibiotic treatment and severity of clinical presentations during cases (Banerjee et al., 2011, 2014; Katz et al., 2013). The review aims to emphasize on the need for molecular and genetic epidemiology of Non-O1/Non-O139 and O1/O139 V. cholerae as a prospective future for understanding the dynamics, spread and control of emerging environmental pathogens in rural communities of African and other continents.

## METHODS

A coordinated and structured review of relevant literatures on the distribution, spread/transmission of cholera cases especially in Southern African countries were accessed. Such relevant published documents from related investigators were recovered from google scholar, Pubmed, Scopus, and Web of Science. Other information collated were used to describe the distribution of toxigenic V. cholerae and steps in investigating the outbreak especially in high risk countries. The data collected includes clinical and environmental reports which might aid in deciphering and/or estimating polymorphism between strains, source, serotype and group.

### Search Strategy

Various search engines including Water Research, PubMed, Google Scholar, and Web of Knowledge (WoS) were sourced between 14/06/2018; 12.08 GMT+2. The title specific search was adopted for the study applying a Boolean according to the search interest (Igere and Ekundayo, 2020; Igere et al., 2022c,d). On Google scholar and Water Research we searched for the situation of water in the Southern and Eastern African countries, civil societal influence on safe water for all and the various driving force for water hygiene/sanitation. On PubMed and WoS we searched for pathogens of the AWD/Cholera, update on the most current state of cholera in African countries, considering them one after the other. We also searched for the various molecular typing methods, the relevance of molecular epidemiology (eg; molecular epidemiology of virulent genes, molecular epidemiology of antibiotic resistant genes, etc), other genetic typing/fingerprinting methods and genotyping techniques. In WoS we accessed basic steps towards studying an outbreak, source tracking of pathogens, other molecular typing methods (eg; Spa typing) that may be applicable in typing emerging pathogens. Other epidemiological and molecular biology techniques text books were also sourced while unrelated documents not showing AWD/Cholera disease information were excluded. We also restricted most area of the study and discussions in this review to the African cholera situation with specific attention on the Southern/Eastern African countries and other continents which are having ongoing cases of AWD/Cholera caused by *V. cholerae* O1/O139 and Non-O1/Non-O139 members.

### Criteria of Inclusion

The published articles which only focus on ongoing cases of AWD/Cholera caused by V. cholerae O1/O139 and Non-O1/Non-O139 members as well as strategies for eradication in clinical and environmental nexus" that have been reported by previous researchers were applied while other non-conforming articles were excluded.

### Data analysis


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All recovered articles were read, summarized, while necessary details related to this study were collated and presented in tables and figures. Such scientific recorded details and narrative summary on the ongoing cases of AWD/Cholera caused by V. cholerae O1/O139 and Non-O1/Non-O139 members and strategies for eradication in clinical and environmental nexus were chronologically arranged as presented below (Igere et al., 2022d).

## Water Situation in African Countries on Cholera/AWD and Global Interventions

Following the World Water Councils report and observation in 2018, it is documented that above 840 million people in the World lack access to safe drinking/domestic water supply (Igere et al., 2022a,b,c; Adams *et al.*, 2018; UNICEF_WHO, 2012, 2017; Cosgrove and Loucks, 2015). The struggle by poor and indigenous communities in Botswana, Zambia, Lesotho, Uganda, Zimbabwe, South Africa, Mozambique, Namibia, Malawi etc for safe water supply and hygiene is on the increase (WHO, 2018). This lack of access to safe domestic water sources and poor hygienic practice has currently been associated with the cholera outbreaks in Zimbabwe (WHO, 2018; Soyapi, 2017a, b; Water Sanitation Program, 2007) and other Southern African countries as shown in fig 1. Strategies to ameliorate and check the outbreaks in these countries include provision of basic hygienic practice, while neglecting molecular epidemiology. It was also observed that within the Southern Africa Development Community (SADC) and other African Countries, various cases were reported for acute watery diarrhea and cholera between 2017 and 2018 in addition to increased mortality rate (Table 1). However there also exist reports of some cholera and acute diarrhea cases arising from diverse bacterial strains which inhabit the water nexus and also spread via water systems causing various clinical cases.

## Cholera and Acute Watery Diarrhea Bacterial Pathogens

According to WHO (2018), Cholera/AWD have arouse major public health concern as it results the death of more than 525,000 children under the age of five, 5% disability associated with life loss and 1.5 billion diarrhea episodes (WGO, 2008; Mathers and Loncar, 2006). AWD/Cholera outbreaks result from pathogens of the enterobacteriaceae family vis; V. cholerae, Salmonellae species, Shigella species, Aeromonas species, Plesiomonas species, Vibrio parahaemolyticus, Campylobacter spp., Yersinia enterocolitica, Escherichia coli O157:H7 its pathotypes etc (Tian et al., 2016; Akhtar et al., 2012; Langendorf et al., 2015). Globally, Cholera/AWD is listed amongst cases of high morbidity and mortality in children of age 0-5 in Sub-Sahara Africa and Asia (Kotloff et al., 2013) and accounts for 11% paediatric death annually (Lanata et al., 2013). Although the pathogens are environment associated and residential flora of the environment, Bhuyan and other groups were able to associate their persistence with paucity of environmental information to substantiate their prevalence in the environment (Bhuyan et al., 2016; Lanata et al., 2013; Nasrin et al., 2013; Liu et al., 2012; Panchalingam et al., 2012; Levine et al., 2012). This has also necessitate the relevance of molecular epidemiology in detection of their virulent genes and appropriate control of such potential pathogens.

## Molecular epidemiology of virulent genes

Studies that seek to reveal the endemicity and pandemicity of V. cholerae often involve collection of environmental specimen/isolates since some of such isolates possess virulence potential for an outbreak (Igere et al., 2022a,b,c,e,f,g; Lindsey et al., 2017; Islam et al., 2013). Some of such virulent indices include ompW, wbeO, wbfO, ctxA, ctxB, hlyA, zot, ace, OmpU, tcpI, toxR, stn/sto, PilE, rstC, acfB, mshA, rstA, rstR, mdh, adk, gyrB, recA, vcsC, vspD, vcsN2, vcsV2, chxA and HA/protease etc, which are involved in the regulation, penetration, phage attachment, adhension, invasion and epidemic virulent dynamics etc. Others are inclusive in serotyping, transmission and persistence in the environment (Bhuyan

et al., 2016). Another aspect that was applied recently is the use of housekeeping genes of multilocus sequence typing technique (MLST) such as adk, metE, chi, gyrB, dnaE, recA, pntA, cat, gyrB, purM, mdh, pgm, lap, rstA, gmd and pyrC (Liao et al., 2018). Applying the various genotyping techniques in both environmental and clinical isolates possess the potential for genetic epidemiology of such pathogens. It may also encourage early field-based detection/confirmation of disease causing strains in related cases and impact suggested experiment-based management/control strides.

## Molecular Epidemiology Of Antibacterial Resistant Genes Amongst Emerging Pathogen

Over the years, V. cholerae have acquired adaptive response to many antibacterial agents and develop resistance to numerous groups of antibiotics. Some of them have shown presence of mobile genetic elements (Igere et al., 2022e,f; Yaovi et al., 2016; Ismail, 2015; Spagnoletti et al., 2012; Islam et al., 2012), while others have shown presence of integrative conjugative elements (Haley et al., 2012) which may smooth the progress of inter- and intra-species dissemination of antimicrobial resistant genes (Taviani et al., 2012). The study of Thapa et al. (2017) revealed that Nalidixic acid, Ciprofloxacin, Co-trimoxazole and ampicilin should not be applied as first line of antibiotics in the treatment of shigellosis owing to the high resistant acquiring nature observed during their study (Table 3). Mortality rate associated with multiple antibacterial resistance is currently on the increase especially amongst low and middle income countries (Lim et al., 2016; WHO, 2014; Laxminarayan et al., 2013). Although antimicrobial chemotherapy/research has shown promises and great relevance in the control and/or treatment of various bacterial implicated infections and diseases, the case is different especially amongst enterocyte infecting pathogens associated with outbreak. This has resulted a quick and sudden emergence of resistant pathogens in the post antibacterial era (extensive drug resistance (XDR) towards pan drug resistance (PDR)). Hence the need for molecular biology based epidemiological technique in other to investigate molecular epidemiology of such resistant genes and updated periodic surveillance of the antibiotic resistant patterns (Igere et al., 2021a,b; Samajpati et al., 2018; Rahimi et al., 2017; Taneja and Mewara, 2016).

## Mode of transmission and spread of cholera

Pathogens that are implicated in the spread and transmission of acute diarrhea (AWD) and cholera eg the Non-O1/Non-O139 and O1/O139 V. cholerae are autochthonous to the environment, where it specifically inhabit coastal water, rivers and estuaries etc (Reimer et al., 2011; Constantin et al., 2008). Notable mode of transmission includes faecal-oral route and person-to-person contact. Poor environmental hygienic practice, lack of clean/safe water, close personal contact and malnutrition are possible influencing factors (WHO, 2017; Jahan, 2016; CDCP, 2014; EFSA, 2008; Niyogi, 2005). Other environmental factors such as temperature, seansonal dynamism (rainfall/ Sunshine), climatic condition and humidity have also been reported as associate (Torres, 2004; Thapa et al., 1995) to the transmission and spread of Cholera/AWD. The persistence, spread and transmission in an environment is an indication of socioeconomic problem and threat to global public health (Jahan, 2016), since it is associated with the social well being and/or behavior of the people in an area or community. Consequently in any environment suspected to harbor the pathogen, when two or more sampling points/sources shows the presence of the pathogen in both culture and molecular detection techniques with varying pathogenic dynamics, there is a likelihood of transmission between these two sources and/or sampling point via a vector of that environmental niche. Various investigators are driving the need for understanding the mechanism of Cholera/AWD transmission especially in the endemic countries with interest of averting any outbreak oriented factors. Such steps are channeled towards modeling the


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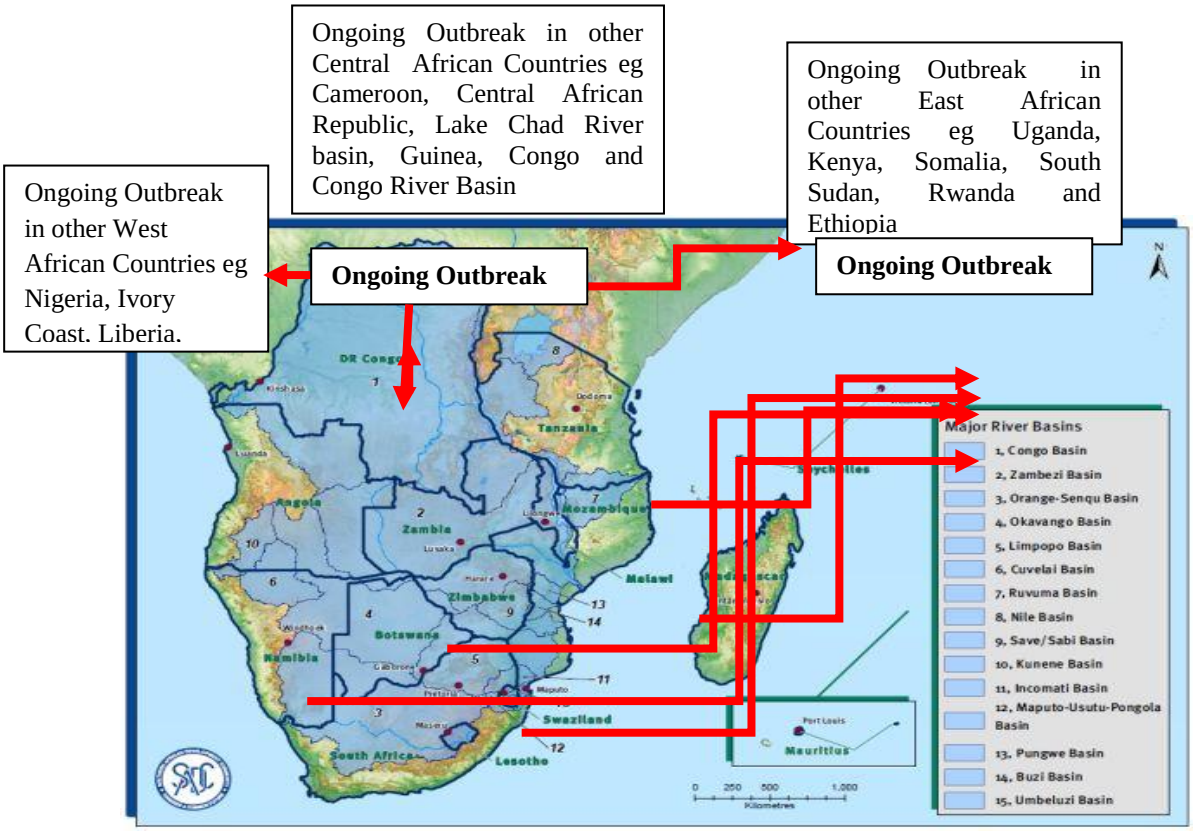
mode/mechanism of cholera/AWD transmission. The figure 2 below shows a chat of transmission in real life with multiple sources carrier of the pathogen.

**Figure 1.** Southern African Development Community (SADC) River basin and Ongoing Cholera/AWD Outbreaks; the impact on safe water (Adopted from SADC,2012; www.sadc.int/themes/natural-resources/water/)




## Modeling Transmission

Disease transmission may be modeled by the application of mathematics, molecular biology, bioinformatics tools, computer science and statistical framework to determine the mobility of pathogens (V. cholerae Non-O1/Non-O139 and O1/O139 strains) within an environment (Mukandavire and Morris, 2015; Fung, 2014). De Maio et al. (2016) applied the Structured COalescent Transmission Tree Inference (SCOTTI) to draw a transmission tree. It is an online free source computer dependent package (BEAST2 software) for phylogenetic studies.

**Table 1.** Southern Africa Development Community and other African Countries case report of Acute Watery Diarrhea and Cholera between 2017 and 2018

| Country | Disease | Year of reporting | Current Situation* | Propable and Total cases reported | Confirmed cases | Number of death recorded | Case Fatality Rate(CFR %) |
|---|---|---|---|---|---|---|---|
| Angola | Cholera | Apr-18 | Ongoing | 917 | 5 | 15 | 1.6 |
| Burundi | Cholera | Apr-18 | Reduced epidemic | 330 | ND | 0 | 0 |
| Congo | Cholera | Apr-18 | Ongoing | 45 | 3 | 2 | 4.4 |
| DR Congo | Cholera/Acute Watery Diarrhea | Apr-18 | Ongoing | 61,860 | ND | 1327 | 2.4 |
| Ethiopia | Acute Watery Diarrhea | Apr-18 | Reduced epidemic | 48,971 | ND | 880 | 1.8 |
| Kenya | Cholera | Apr-18 | Ongoing | 23,639 | 121 | 398 | 1.7 |
| Malawi | Cholera | Apr-18 | Intervention | 918 | 195 | 30 | 3.3 |
| Mozambique | Cholera | Apr-18 | Ongoing | 2329 | ND | 5 | 0.2 |
| Namibia | Cholera | Mar-18 | Reduced epidemic | 1 | 1 | 0 | 0 |
| Nigeria | Cholera | Mar-18 | Reduced epidemic | 210 | 2 | 16 | 7.6 |
| Rwanda | Cholera | Mar-18 | Ongoing | 5 | ND | 0 | 0 |
| Somalia | Cholera | Mar-18 | Ongoing | 2,267 | 312 | 9 | 0.4 |
| Somalia | Cholera/Acute Watery Diarrhea | May-18 | Ongoing | 78596 | 312 | 1118 | 1.4 |
| South Africa | Cholera | Mar-18 | Checked | 1 | 1 | 0 | 0 |
| South Sudan | Cholera | Mar-18 | Ongoing | 20, 438 | ND | 436 | 2.2 |
| Sudan | Choleta | Mar-18 | Reduced epidemic | 77 | ND | 1 | 1.3 |
| Tanzania | Cholera | Apr-18 | Ongoing | 30, 323 | ND | 499 | 1.6 |
| Uganda | Cholera | Apr-18 | Ongoing | 2, 224 | 24 | 47 | 2.1 |
| Zambia | Cholera | Apr-18 | Ongoing | 5706 | 565 | 113 | 2 |
| Zimbabwe | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 |

*Recorded ongoing cases may change with time as reports are documented (Adopted from WHO-AFRO, 2018, 2017)

## Source Tracking Cholera and Acute Watery Diarrhea Outbreaks

Since 1817, cholera has been a major public health concern both to non-governmental organizations, civil societies and the government. Tracking the source of the pathogens entails real crude sampling of various sources. In 1854, John Snow embarked on similar adventure, which involves lot of experimental analysis of sample, until the confirmation of the cholera pathogen habitat to coastal public tap water and environments (Bill and Melinda, 2010; Lidwell, 1987; Snow, 1854, 1855). It was Snow's work that heralds the basis as well as the foundation of epidemiological studies today. Source tracking is an experiment based detection techniques that employs an environmental index case or first case patient or source (an individual indicator) that call the attention of investigators to a study. The activities and contacts (person-to-person, person-to-material, person-to-food) of the case index is then mapped or traced backward with the hope of reducing spread until the contacts of the index case and activities are investigated and ruled out of any association with the disease case (Snow, 1855). This is done in favour of the Germ theory of diseases that contact with contaminated materials ensures transmission of diseases. Currently, with advances in technology, there is a twist in the cartel as the computer is been applied to walk through the process of tracing the origin of an infection and detecting using molecular biology techniques. This may not be different if molecular and genomics epidemiology is applied in the ongoing pandemic situation of cholera/AWD in world.

## Other typing strategies of the strains

Typing of such strains may encompass detection of specific strain related biomolecules such as phage, plasmid (extra-chromosomal DNA), and othet biological agents which some strains acquire or possess due to their previous exposure and habitat. The basic applied typo-genomic strategy may depend on the target biomolecule which needs to be studied or detected. Generally the strategy may include molecular typing or fingerprinting in any environment as well as reported case.

## Molecular Typing

Molecular typing of pathogens/genes or microbial fingerprinting is an essential tool in the molecular epidemiology of both outbreak and non-outbreak associated pathogens. Its relevance is derived from the high discriminating power reported from it, in various studies in (Hossain et al., 2018; Liao et al., 2018; Sabat et al., 2013). epidemiological surveillance, prevention and control of most pathogens of human, animal, agricultural and environmental relevance. It is applied systematically in source intervention studies, genetic analysis and evolutionary studies despite some observed and reported demerits in interpretation of data (Rahaman et al., 2015; Mishra et al., 2011; Arti et al., 2011; Bhowmick et al., 2007). Molecular typing has been applied in pathogen characterization, pathotyping or virulence typing, identification of clonal complexity and/or relationship and source tracking of outbreak associated or non-outbreak associated pathogens such as V. cholerae Non-O1/Non-O139 and O1/O139 strains. According to Sammarco et al. (2014), epidemiologically relevant typing methods sub-divided into four principal sections: the differentiation and identification of polymorphic restriction sites in extra-chromosomal DNA/chromosomal DNA, amplification of specific gene of interest, restriction digest analysis and sequence genotyping analysis. Its application in emerging pathogenic bacteria is a noteworthy aspect of Genomic/molecular epidemiological studies especially on enteric pathogens, nosocomial pathogens and multiple antibacterial pathogens. The application is aimed at appraising epidemiological surveillance and understanding the disease distribution/pathogenesis of the pathogen (Shokoohizadeh, 2016; Hallin et al., 2012).

**Schematic Cycle In Mode Of Spread/Transmission Of Pathogens**

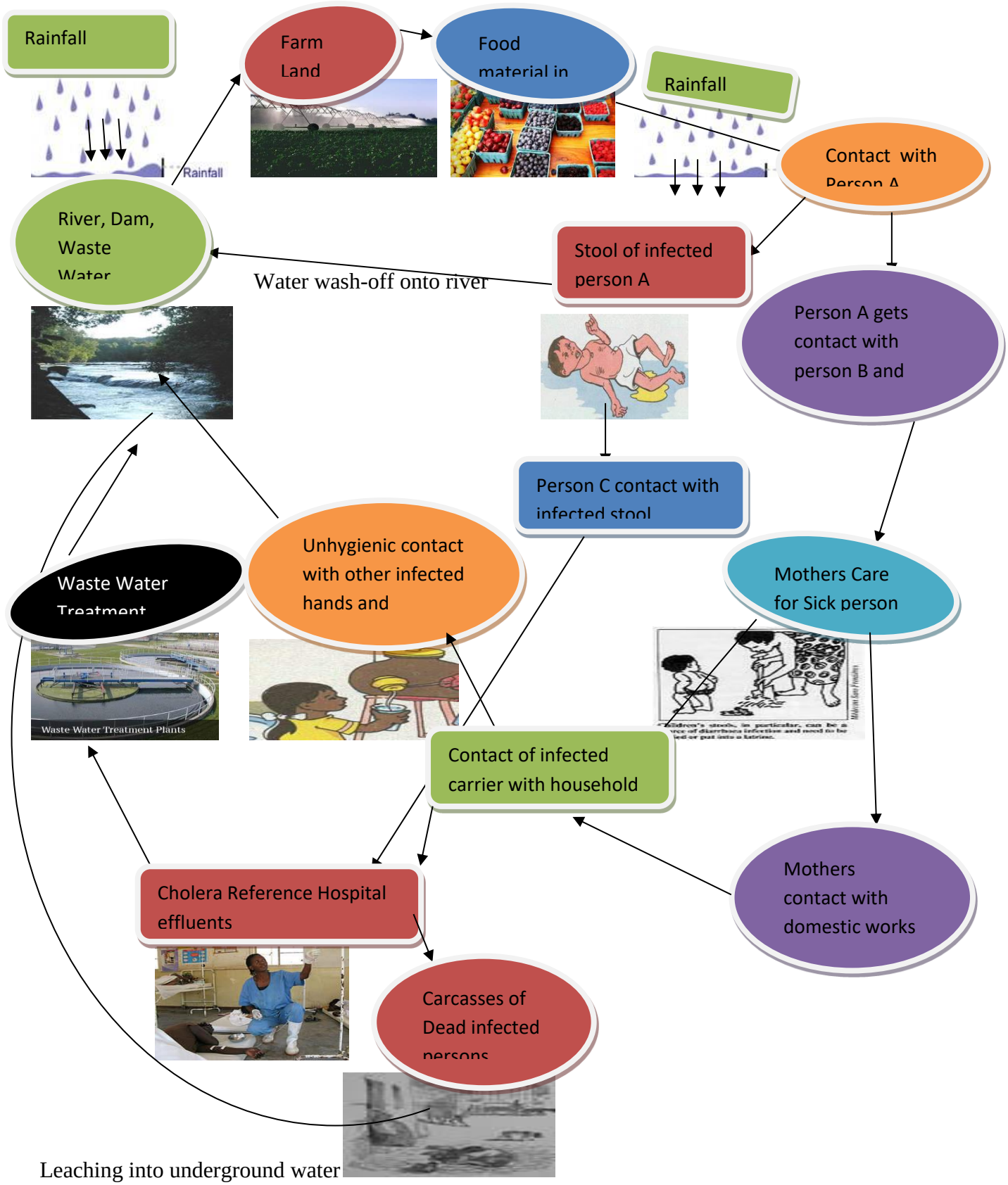



**Figure 2.** Chat showing Transmission/spread of cholera in a community

## Molecular typing methods for outbreak investigation and transmission control

Molecular typing techniques applied for investigating outbreaks should possess excellent typeability characteristics and possible discriminating power in other to determine the divergence observed in the epidemiological surveillance studies of emerging pathogens. The methods should be non-expensive, easy to perform/interpret, high reproducibility, rapid, portable/database assessable, stable with time, applicable with international standard and able to decipher relatedness and polymorphism amongst strains of person-to-person (local) or regional transmission (Sabat et al., 2013; Van Belkum et al., 2007; Struelens, 1996) with a view to controlling transmission. Other target specific and in-expensive techniques of relevance in epidemiological surveillance include:

### Pulse Field Gel Electrophoresis (PFGE)

It is usually employed in North America as the typing method for molecular epidemiological studies. It is a nucleic acid separation technique used to discriminated pathogens genes and their gene diversity after a prior endonuclease treatment (Ranjbar et al., 2014; Mishra et al., 2011).

### Ribotyping

This is a molecular typing method applied mainly in Europe to access the variation of intergenic spacer region (ISR) around ribosomal RNA segment (16S and 23S). The choice of ribotyping technique recently is based on the recently observed global/international standard relevance for comparison and interpretation of ribotype data (Arcenas, 2017; Huber et al., 2015).

### Variable Number of Tandem Repeats Typing (VNTRs)

The typing technique was designed after the discovery of numerous genomic investigation of the prokaryotes genome. It was observed that the bacterial genome consists of numerous short nucleotide repetitive elements of about 53 basepair size which may change both in number and sizes during DNA replication and may result errant and changes in the nucleotide sequences (Sabat et al., 2013; Crawford et al., 2013; Crawford & Nickerson,2005).

### Multilocus Sequence Typing (MLST)

It involves targeting the nucleotide sequences of multiple genes locus ranging from seven to twelve with band size of 400 – 500 base pair. Each of these genes of interest are designated as sequence type (ST), and they are assigned with an arbitrary numerical code. Major advantage of the technique is it's high reproducibility and easy comparability of nucleotide sequences amongst inter laboratory reports as observed in the data base (Lam et al., 2012; Guigon et al., 2008; www.mlst.net).

### Spa typing

This typing technique is employed basically for Staphylococcus aureus protein A gene region (spa). It is also referred to as the single locus sequence based typing technique (SLSBT). The method involves sequencing the polymorphic X region of protein A in the pathogen (pathogenic studies) by targeting well conserved region of variable number of 24 base pair repeats it is relevant due to its reproducibility, portability, rapidity and micro/macro polymorphic dynamics which enable their application in both local and global molecular epidemiological studies (Mayerhofer et al., 2015; Hallin et al., 2009).

### Haplotyping

This typing method can enhance the identification of various polymorphic sites applied in the investigation of emerging pathogens such as V. cholerae. It is observed that the genetic heterogeneity of V. cholerae pathogenic strains and their respective virulence determinants are currently unpredictable in natural habitat (Zojer et al., 2017).

## Whole Genome Sequencing

Over the years, advances in research has impacted on applicability and accessibility of molecular typing technique. Recent advances were directed at improved speed and entire gene coverage increasing the need for an whole genomic sequence study of bacterial population. Whole genome sequencing facilitates the study of genomic heterogeneity including haplotypes, VNTRs, short tandem repeats (STR), MLST, PFGE and other alleles of reduced detectable frequency in various molecular techniques (Zojer et al., 2017; Bentley & Parkhill, 2015; Parkhill & Wren, 2011).

## Why Employ Molecular Typing or Genotyping

Molecular typing has been used as a tool to confirm and interpret findings from studies on epidemiology. This is so as most bacterial pathogens now presents with identical clinical symptoms, hence confirming the specific pathogen which is associated with a disease case requires genetic or genomic typing (Mikosz et al., 2014; Smith et al., 2013; Fanfair et al., 2012).

### The Role of Phylogenesis in Molecular and Genomic Epidemiology

Phylogenesis has been reportedly used in pathogen evolutionary history and bacterial relationship studies to trace outbreak or disease transmission over a period of time. It is analysed by drawing developed from either whole/partial 16S rDNA gene sequence data which are annotated, assembled and used in determining the relationship amongst pathogens to produce clades of common ancestor (monophyletic) and unrelated strains (polyphyletic).

## Prospects of Molecular Epidemiology for Future Application

Although WGS has proven out-right relevance in the investigation of outbreaks and source tracking of outbreak, its application in multiple sequencing centre's for surveillance studies is greeted with numerous challenges. These are accurate nomenclature/identity definition for WGS-derived pathogen, absence of standard bioinformatics pipelines, disparity in WGS-sequencing platforms, older typing technique comparability with WGS and translation/interpretation of genomic sequence data to useful information for decision making in public health system and civil society.

Other challenges include sequence dissimilarity amongst developing continents or countries who apply WGS technology in public health laboratories must be avoided by investing at national levels on the routine application of WGS by training experts and providing resources in public laboratories and/or training hospitals (Revez et al., 2017a,b). In achieving these objectives, the Economic Cooperation among Developing Countries (ECDC) was instituted and it provides with Member States an expert opinion on WGS for surveillance studies in public health system.

Avoidance of these challenges would encourage appropriateness in the application of WGS and in the future enhance application of molecular epidemiology of environmental potential pathogens, virulent genes, antimicrobial resistant genes and other emerging dynamics of the Vibronaceae family. It will also provide useful information to deciding the treatment/management strategies and antibacterial stewardship programs in endemic environments.


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**Table 3.** Multiple Antibacterial Resistant Markers Amongst Vibrio cholerae

| Pathogen Serotype | Disease Source | Resistant Markers | Period | Country | Reference |
|---|---|---|---|---|---|
| O1 ElTor | Cholera (Clinical) | T, Step, Na, CH, Imi | 2007 – 2008 | Kenya | Saidi *et al.*, 2014 |
| Non-O1/Non-O139 | " | Amp | " | " | " |
| O1 | Cholera (Clinical) | Amp, Chl, Strep, T | 1993 – 1998 | Ethiopia | Scrascia *et al.*, 2009 |
| O1 | Clinical | Pen, strep, Na, Ery, Tet, T | " | " | " |
| O139 | Clinical | CH, Tet, Ery, Strep, T, Cephalo, Plasmid | 1993 – 2009 | China | Wang et al., 2015 |
| Non-O1/Non-O139 | Clinical | ESBL, Imi, Amp, Cef, Ceft, Ert, Mero | 2013 | France | Aberkane et al., 2015 |
| O1 | Environment/Water | Na, T, Am, Tet | 2012 | China/Hong kong | Po et al., 2015 |
| Non-O1/Non-O139 | Environment/Clinical | NDM-1 | 2010 – 2013 | Vietnam | Diep et al., 2015 |
| O1 ElTor | Envirinment/Clinical | T, Am, Strep, Tet, Ch, Plasmid | 1994 | Rwanda | Carraro et al., 2014 |
| Non-O1/Non-O139 | Clinical | Sulfamet,Tet,Ch,T,Strep, Spec,Am,Na,Azit,Nit | 2010 | Haiti | Carraro et al., 2016 |
| O1 El Tor, Ogawa | Clinical | Sulfamet,Tet,Ch,Am,Na,Cot, Nit, Azith, T, Dox | 2012 – 2015 | Mozambique | Dengo-Baloi et al., 2017 |
| O1 | Clinical | Cot, T, Ery, Tet, Cip, Doxy | 2010 – 2012 | Ghana | Kuma et al., 2014 |
| O1 El Tor, Ogawa | Environmental | Sulfamet, T, Na, Ery, Ch, Tet, Amp | 1997 – 2012 | DR Congo | Miwanda et al., 2015 |
| O1 Atypical El Tor | Clinical | Cip, Ch, Imi, Na | 2009 – 2010 | Nigeria | Marin et al., 2013 |
| Non-O1/Non-O139 | Clinical | Na | " | " | " |
| O1 Ogawa/Inaba | Clinical | Cot, Na, Amp, Fz | 2008 – 2011 | Iran | Salimi-Khorashad et al., 2012 |
| Non-O1 Ogawa | Environment/food | Amp, Strep, Kan, Sulfamet-T, Ery, Tet, Cip | 2012 | Indonesia | Waturangi et al., 2013 |
| O1/O139 Ogawa | Clinical | Co,Fz, Na, A, Nit, S, T | 2004 - 2013 | India | Pal et al., 2018 |
| O1/O139 Ogawa,Non-O1/-O139 | Clinical | T, A, C | 2011 - 2014 | Ghana | Eibach et al., 2016 |
| O1 Ogawa, Non-O1/Non-O139 | Clinical | Na, Am, A, C, Cef | 2011 - 2015 | North India | Uppal et al., 2017 |
| O1 El Tor, Ogawa | Clinical | T, Ch, Doxy, Tet,Fz | 1990 - 2004 | Zambia | Mwansa et al., 2007 |
| O1 Inaba/Ogawa | Clinical | Co, Cm | 1993 - 2005 | Pakistan | Jabeen et al., 2008 |
| O1 Eltor ogawa | Clinical | Tet, Qu, Cm, Co | 2002 - 2004 | Mozambique | Mandomando et al., 2007 |
| O1 El Tor, Ogawa/Clas CTXϕ | Clinical | Co, Na, Spec, Sm, Sxt, Nitrofi | 2004 | India | Goel et al., 2010 |
| O1 El Tor | Clinical | Co | 2004 2006 | Senegal | Manga et al., 2008 |
| O1 | Clinical | SXT, Amp | 2004 - 2005 | Cameroun | Ngandjio et al., 2009 |
| O1 Inaba | Clinical | Co, Cm, Amp, Ery, Tet, Cpr | 2006 - 2008 | Ethiopia | Abera et al., 2010 |
| O1 Eltor Inaba | Clinical | SXT, Sm | 2006 2007 | Namibia | Smith et al., 2008 |
| O1 Eltor and Ogawa/Inaba | Clinical | Fz, SXT | 2009 | Zimbabawe | Islam et al., 2009 |
| O1 | Clinical | SxT | 2006 | Ghana | Opintan et al., 2008 |
| O1 El Tor,Ogawa | Clinical | ESBL,Na,Oxa-,T,Sul,Ch,Kan,Strep,Ery,Amp,Tet, Cef | 2008 - 2009 | South Africa | Ismail et al., 2013 |
| O1/Non-O1, Ogawa | Clinical | Amp, Kan, Strep, Sulfonamige, Tet | 1983 - 1990 | Somalia | Coppo et al., 1995 |

## CONCLUSION

Since the discovery of V. cholerae (Non-O1/Non0O139 and O1/O139 members) as pathogen of clinical relevance in cholera and AWD cases, numerous advances by investigators havd included molecular techniques. Currently, there is a paradigm shift from microbial genotyping to Whole Genome Sequencing (WGS) using Next-Generation Sequencing (NGS) technique. It is envisaged that whole Genome sequencing (WGS) would transform and revolutionize molecular epidemiology owing to the great prospects presented by NGS. It would influence public health surveillance, source track pathogen and impact investigations of outbreaks. It will also enhance laboratory based antimicrobial resistance profiling and pathogen risk assessment. The application of pathogen sequence phylogeny may also influence studies on multiple antimicrobial resistance and determining the spread of such resistant nature amongst pathogens, clonal complexity and association to outbreaks of pathogens. A re-directed attention or interest on molecular epidemiology would help to build defense capacity against any imminent outbreak of V. cholera or its epidemic as well as any control dependent problems.


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### Conflict of Interest

No conflict of interest was declared

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