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Treatments of cholera, their restraints and the necessity for simple prevention strategies: a comprehensive review

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Abstract:

Cholera, caused by waterborne bacteria Vibrio cholerae, has been proved to be one of the most important concerns in both developing and under developed countries with inadequate access to safe drinking water and sanitation. This gram negative facultative anaerobe, upon ingestion, accumulates in human gut and the cholera toxin exerts its effects on enterocytes integrity, ion channels, causing drastic loss of salt and water, ultimately resulting in death if not or inefficiently treated. Its colonization via colonizing factors and toxic activity of the multi-subunit cholera toxin has been the centre of interest to the scientists since the emergence of the disease in modern history. Based on the biochemistry of cholera toxin and the colonizing factors, several interventions have been formulated so far, such as rehydration therapy to combat liquid loss, homeopathy treatment to reduce stool volume and diarhhoeal episodes, administration of antibiotics and bacteriophage as vibriocidal agents, zinc supplementation for restoring intestinal integrity as well as antibody production, vaccination strategy involving whole bacterial cells and cholera toxin virulent subunit. A number of clinical trials have also reported the efficiency of these strategies. But unfortunately, none of these are without limitations, which in certain occasions even overshadow the efficacies. The primary objective of this review is to put forth the formulation philosophy and outcomes of these disease combating measures and their severe clinical, economical and epidemiological parameters-based limitations. This review also emphasizes on the idea of directing research as intense as treatment researches, towards prevention strategies such as water treatment at storage as well as at point of use level or formulation of simple, universal biochemical interventions, which certainly will be more beneficial and helpful for the suffering people at any given set of parameters and settings.

Keywords:

Antimicrobial; cholera; homeopathy; oral rehydration solution; water treatment; zinc supplementation

1. Introduction

One of the most primitive bacterial diseases, which is yet to be said 'managed' with confidence, is Cholera, exclusively caused by the bacterium *Vibrio cholerae*, a gram negative, rod shaped, and facultative anaerobe. Modern history has documented the occurrence of cholera since 1817 emerging from Ganges delta of Indian Subcontinent spreading still Southeast Asia, but is thought to exist even in long past (Joachim and Karl, 2002). Till date, there are eight severe pandemics of cholera reported, leaving almost not even a single continent. Decades of research on the disease has revealed handful of information regarding the causing agents, its genetic constituents and tricks to lead a human to severe health concerns, even death. Based on these several novel findings, there are number of combating measures formulated till date and research works going on for a cent percent efficacy of these treatments. The most efficient treatments suggested are rehydration therapy, antibiotic treatment, zinc supplementation, vaccination and few more. Unfortunately all the mentioned measures exist with their own limitations.

In order to understand the philosophies of the formulated treatment measures and the reasons for their ineffectiveness under certain circumstances, it is much needed to understand the mechanism of action of the microbe. An

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understanding of action principle of the bacterium will certainly help in assessing the treatment fundamentals. Though there are several strains of the bacterium for example, O1 Classical, E1Tor, Inaba, Ogawa, O139, that have been emerged so far and have their uniqueness in structure, antigen possession and thus interacting molecules, still, they work on same basic principle.

2. Mechanism of action

The complete pathway of the infection commences with the ingestion of infected food or water. Upon intake of contaminated drinking water and food the bacterium make its way to intestine where it colonize and produces toxin material, finally producing the symptoms, if neglected, leading to death.

Colonization: The most important factor for colonization of the bacterium in the human gut is the TCP (Toxin Co regulated Pilli), ACF (Accessory colonizing Factor), some other gene products for example, ToxR,ToxS,ToxT , membrane porins, O antigen of LPS (Lipopolysaccharides) (Shah *et al.,* 1998) and so on. TCP is a polymer made up of TcpA protein monomer which is also the receptor for CTXϕ bacteriophage. The gene products are required for controlled TCP transcription. Membrane porins for example, OmpU indirectly helps in colonization by protecting the cell from damage by bile salts and organic acids.Besides these factors, the bacteria also express a Glucosamine Binding Protein (GbpA) to bind to the glucosamine subunit of mucin, the abundant constituent of mucus lining the intestinal wall.

Toxin infection: Cholera Toxin (CT), also known as Choleragen, is the critical component for the infection and the onset of the disease. The toxin is encoded by *ctxAB* gene which is in turn is a part of CTX element. CT is made up of one A subunit and five B subunits. CT action mechanism starts with the integration of the toxin with the ganglioside receptors (G_{M1}) expressed on the intestinal epithelial cells via a tryptophan residue of B subunit. This attachment triggers the endocytosis of the toxin while the subunit A gets cleaved and forms the active A_1 subunit. A_1 in turn goes into the cytoplasm and attaches to the Gα subunit of the G protein and locks it in a GTP bound state which the active state of this particular protein is. This incidence in turn keeps the adenylate cyclase activated producing cAMP in excess. Higher cAMP level activates the Cystic Fibrosis Conductance Regulator (CFTR) causing dramatic efflux of sodium ions and hence water. Figure 1 explains the mechanism of action of cholera toxin.

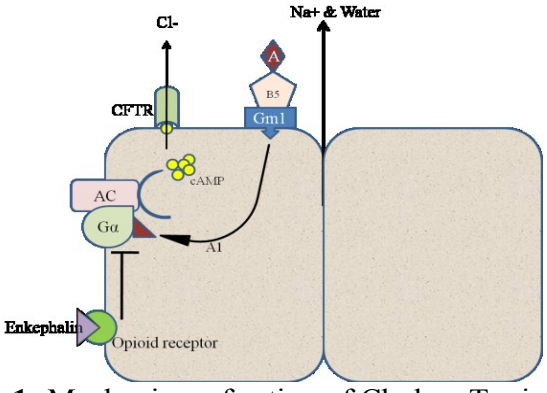

Figure 1: Mechanism of action of Cholera Toxin (CT).

3. Treatments

Several treatment measures have been formulated and studied so far. Though these techniques have demonstrated considerable efficiencies but are yet to overcome their limitations of efficacies, cost-effectiveness, and availability. The available in practice treatments along with the curing strategies in infancy and identified limitations are summarized further.

3.1Rehydration therapy

The history of rehydration therapy can be traced back in 1831, when O'Shaughnessy analyzed cholera patient's blood and stool, concluded the deaths occurring due to

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water and salt loss from body. He further recommended injection of salt intravenously to reverse the effects (Guerrant *et al.,* 2003). Rehydration therapy has been implemented based on the fact of dehydration caused by rapid water loss from the intestine, which in turn can cause many complicationsfor example, low blood pressure, low blood volume, loss of elasticity of muscles and so on. Hence, to overcome these, Oral Rehydration salt has been applied to the patients based on the extent of dehydration, age and body weight. Table 1 outlines the criteria and dosage of ORS as prescribed by WHO.

Severity	Symptoms (WHO)		Type of fluid	Dose quantity
No dehydration	Conditions Eyes Thirst Skin pinch	Well, alert. Not sunken. Normal intake. Heals fast.	ORS	• <2 yrs: 500ml/day • 2-9yrs: 1L/day. • Adult: 2L/day.
Some dehydration	Conditions Eyes Thirst Skin pinch	Restlessness. Sunken. Drinks eagerly. Heals slowly.	ORS	• <4months: 200-400ml/day. • 4-12 months: 400-600ml/day. • 1-2yrs: 600-800ml/day. • 2-4yrs: 800-1200ml/day. • 5-14yrs: 1200-2200ml/day. • >14yrs: 2200-4000ml/day.
Severe dehydration	Conditions Eyes Thirst Skin pinch	Lethargic. Sunken. Not able to drink. Heals very slowly.	Intravenous saline + ORS (if able to drink)	• <12months: 30ml/kgBW (1hr) + 70ml/kgBW (5hrs). • >1yrs: 30ml/kgBW (30min) + 70ml/kgBW (2.5hrs)

Table 1: Criteria and dosage of Oral Rehydration Solution prescribed by World Health Organization (WHO).

These ORS constituents are primarily water, sodium, potassium, Glucose and so on. Potassium was excluded primarily when Sir Leonard advocated the Rehydration solution, but a study Govan andDarrow in 1946 demonstrated a fivefold reduction in the mortality rate in cholera patients upon its use (Watten and Philips 1960), since then the potassium has been reincluded in the composition. The most widely recommended ORS solution has an osmolarity of 311 mOsm/L, but recent studies have shown a better efficiency of reduced osmolarity ORS (245 mOsm/L), a study by Pulungsih*et al.,* has suggested that Reduced osmolarity ORS more efficiently reduces the vomiting and increases urine volume when compared to Std WHO ORS (Pulungsih *et al.,* 2006). In addition to the standard ORS composition any additional adsorbent (for example, charcoal) used showed negative effects in regard to diarrheal duration and duration of bacteria excretion (Sack *et al.,* 1970).

Besides the ORS, intravenous injections of the rehydration solutions are also used in case of severe dehydration. In case of unavailability of the same, the ORS solution can be administered by naso-gastric tubes.A study by D. Mahalanabis in 1972, has showed that Ringer's lactate alone as intravenous supplemented with standard ORS gives desired result (Mahalanabis *et al.,* 1972). Another form of ORS, i.e. Polymer Based ORS, contains whole rice amylopectins or other polymers (maize, sorghum, wheat and so on.) to facilitate slow release of glucose enhancing the reabsorption of water and electrolytes. Individual Studies by Fontaine *et al.,* and Gregorio *et al.,* have shown better results in adults' for example, shorter duration of diarrhea, lower total stool volume (Fontaine *et al.,* 2007; Gregorio *et al.,* 2009). Similar low

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osmolarity, WHO- ORS , electrolyte/mineral solution based new rehydration solution has also been mentioned for treating malnourished children, designated as ReSoMal by WHO (WHO 2003) .In order to make the therapy more efficient, Ramakrishna *et al.,* suggested an ORS complemented with amylase resistant starch which produces more amount of indigestible carbohydrates and more amount of short chain fatty acids which in turns facilitates increased fluid absorption and reduced fecal fluid loss compared to standard ORS therapy (Ramakrishna *et al.,* 2000).

In spite of having great effectiveness of this treatment strategy, there are drawbacks such as adequate availability of the ORS for cholera in developing as well as the developed countries for example, US during the most severe cholera outbreak of last century in 1992, a review made by Besser and colleagues (Besser *et al.,* 1994).This particular therapy is yet to attain 100% effectiveness in severe cholera patients as it can only reduce the mortality rate efficiently in mild and moderate diarrhea.

3.2 Antimicrobial therapy

Since the eve of the treatment studies of cholera, antimicrobial therapy has been proved to be one of the most efficient along with ORS in combating cholera. Number of trials has proved the benefits of antibiotics in treating the disease. Antibiotics can significantly reduce the stool volume by 8-92%, diarrhea duration by 50-56% and fluid loss when administered along with intravenous rehydration solution compared to only injections (CDC 24/7 2013).

There are several antibiotics referred by WHO and few other Health organizations like, International Centre for Diarrheal Disease Research , Bangladesh, Pan American Health Organization used for treatment of cholera, namely, Tetracycline, doxycycline, ciprofloxacin, Erythromycin, furazolidone and so on.

The basic mechanism of antibiotic action is to kill the microorganism by means of cell destruction, halting metabolic activities and so on. For example, tetracycline and doxycycline act on 30S subunit of ribosome, and thus preventing protein synthesis. Each antibiotic molecule has unique mechanism of killing a microorganism few of which are represented in Table 2.

In addition to the above mentioned antibiotics, several studies have suggested administration of azithromycin as an effective treatment where azithromycin has showed lower diarrhea and vomiting when given 1.0 gm in adult patients affected by both O1 and O139 strains of *Vibrio cholerae* (Saha *et al.,* 2006, Nelson *et al.,* 2011). Administration of Norfloxacin has been suggested by a study conducted by Bhattacharyaand colleagues during a cholera outbreak study at Kolkata, India (Bhattacharya *et al.,* 1990).

Antibiotic	Class	Mechanism	Target site
Tetracycline	Tetracyclines	Protein synthesis inhibitor	30S subunit of Ribosome
Doxycycline	Tetracyclines	Protein synthesis inhibitor	30S subunit of Ribosome
Erythromycin	Macrolides	Protein synthesis inhibitor	50S subunit of Ribosome
Polymyxin B	Polymyxin	Cell lysis	Cell membrane
Ciprofloxacin	Quinolones	DNA replication inhibitor	Topoisomerase II and IV

Table 2: Examples of antibiotics and their action site and mechanism.

Certain studies have also depicted the efficacy of tetracycline and inefficiency of furazolidone in Bangladesh (Rabbani *et al.,*1989), as well as tetracycline's efficiency over 200 mg single dose doxycycline, though 300mg single dose doxycycline has shown same efficiency as tetracycline (Alam *et al.,* 1990; De *et al.,* 1976), increment in the amount of tetracycline by 2 or 3 times than the standard amount did not enhance the therapeutic result significantly

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(Lindenbaum *et al.,* 1967). The effectiveness of tetracycline (500mg) along with co-trimoxazole has also been reported by Grados et.al,1993 in Lima, Peru among adults against O1 strains which indicated the susceptibility of the strain in that region as resistance to tetracycline was already been reported in Mumbai, India by the time (Grados*et al.,* 1993). Use of another drug i.e. chloropromazine was also found to be effective in lowering ORS failure by 50% among children with severe cholera, the drug was not recommended by the investigators as it was ineffective in case of less severe cholera and failure of ORS was rare in case of severe cholera(Islam *et al.,* 1982). Efficiency Ciprofloxacin has also been documentedin Peruvian adults in 1994-95 (Gotuzzo *et al.,* 1995), in fact Ciprofloxacin has proved to be efficient in treating cholera against tetracycline-resistant vibrios (Khan *et al.,* 1995). Single 400mg dose of furazolidone has also been suggested by Choudhuri and colleaguesfor treating cholera and can be given advantage over other antibiotic agents for its lesser cost (Choudhuri *et al.,* 1968). Nicotinic acid also showed effectiveness in reducing the intestinal secretion when administered in 2mg amount (Rabbani *et al.,* 1983).

Though antibiotics have proved to be an important agent for treating cholera, there are several limitations even in this therapy. These can be listed as:

Lack of specificity: Antibiotics are indeed non specific, to be precise; antibiotics cannot be targeted towards a particular bacterium. As a result it can harm or kill other bacteria which can be beneficial to the body.

Sole treatment: antibiotics cannot be used as a sole treatment of cholera. These agents must be used along with the rehydration salt solutions which actually complement each other in resolving the cholera symptoms. In several cases, doxycycline co-administered with rehydration solution has stabilized the severe cholera patient.

Antibiotic resistant bacteria: The most important problem encountered by the antibiotic treatment is the emergence of antibiotic resistant bacteria. There are number of studies depicting the emergence of *V.cholerae* strains which are resistant to many widely used antibiotics for example, nalidixic acid, sulfizoxazole (Nelson *et al.,* 2011). In 1994, in East Zaire emergence of a *V.cholerae* strain occurred which was even resistant to the tetracycline and doxycycline, the most promising antibiotic against cholera (Siddique *et al.,* 1995), a study conducted byDas and Gupta over a period of 8 years in Delhi has reported the emergence of O1 and O139 serogroup of *V.cholerae* to be resistant highly against nalidixic acid, furazolidone (Das and Gupta, 2005). Few examples of the emergence of antibiotic resistant cholera pathogen are tabulated in table 3.

Antibiotic	Strain	Country 'year
Chloramphenicol	O1inaba/ogawa	Pakistan' 1993
	O1,non O1	Indonesia' 1995
Ciprofloxacin	O1 E1 tor Ogawa	India'1999
	O1	Bangladesh' 2002
	O1 E1Tor Inaba	Iran' 2005
Doxycycline	O1 E1Tor Inaba	Iran' 2005
Floroquinolone	O1,O139	India' 2002
Tetracycline	O1 E1 Tor Ogawa	Mozambique' 2002
	O139	India' 2002
Nalidixic acid	O1, O139*	India 1992-2000
Furazolidone	O1, O139*	India 1992-2000

Table 3: Few of the antibiotic resistant strains emergence and their origin.

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Kitaoka et.al.2011 *Das and Gupta 2005.

Most studied mechanisms involved in these antibiotic resistance mechanisms of the bacteria include the involvement of efflux pumps, genetic mutation, conjugative plasmids, STX elements.

Efflux pumps: *Vibrio cholerae* predominantly utilizes efflux pumps to get rid of the antimicrobial agents such as dyes, detergents and antibiotic drug molecules. These efflux pumps are energy driven either by ATP hydrolysis and Proton motif forces (H^+ / Na^+ gradients).MATE (Multidrug and Toxic Compound Extrusion) and MFS (Major Facilitator Superfamily) are the two most important PMF driven efflux pumps. ATP driven pumps include VcaM. Bacterial efflux pumps responsible for the pumping out of the antibiotic drug molecules and thus, rendering them resistant to antimicrobial action explained in figure 2.

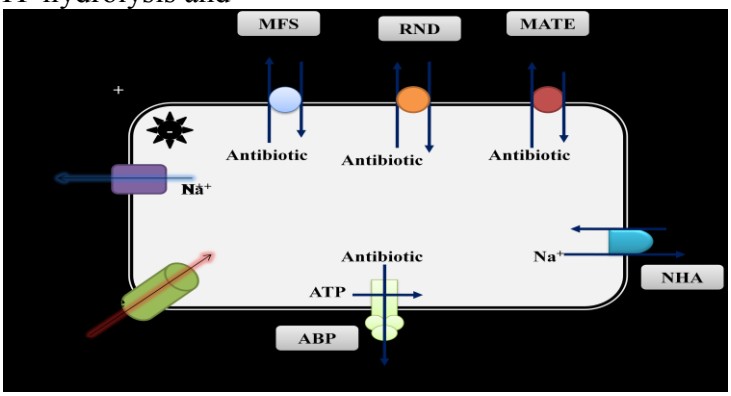

Figure 2: Bacterial efflux pumps responsible for the pumping out of the antibiotic drug molecules and thus, rendering them resistant to antimicrobial action.

Spontaneous mutation: Chromosomal mutations might also contribute to antibiotic resistance. Mutation of cell wall synthesis inhibits the action of alafosfalin which actually targets the bacterial cell wall, another example in *V.cholerae* is mutation in the Topoisomerase gene (gyrA,parC) which in turn inhibits the action of Quinolones which impairs chromosomal replication, DNA stabilit (Kitaoka *et al.,* 2011, Kim *et al.,*2010).

Integrons: These are the naturally occurring gene acquiring systems that facilitate the uptake and integration of exogenous genetic element into the bacterial genome.This particular element essentially contains three components. An *intI* gene encoding integrase, an attachment site *attB* where the exogenous element gets integrated via site specific recombination and a promoter in order to carry out the exogenous gene transcription. These elements play a crucial role in antibiotic resistance because they carry the resistance genes and are associated with the mobile genetic elements occasionally (Ghosh and Ramamurthy, 2011).

STX element and conjugative plasmids: Resistance to antimicrobial agents can also be conferred by the horizontal transfer of certain genetic elements like, STX elements which are actually mobile genetic element belonging to the class of Integrative conjugative elements (ICEs)The exchange of these elements and the conjugative plasmid acts based on the principle that upon conjugation both transfers these genetic element to the no bearing strain and

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makes them gain these element which confers resistance against antibiotics. But unlike the plasmids, these STX has to get integrated to the bacterial chromosome in order to get replicated and expressed at specific attachment (*att*) sites.There are few examples of *V.cholerae*O1having the capability of resisting antibiotics such as, trimethoprim, streptomycin acquired by horizontal gene transfer through natural spread (Kitaoka *et al.,* 2011).Due to these reasons antibiotic treatment has not been prescribed by WHO for the treatment of cholera as these limitations may give rise more serious problems in future.

3.3 Rececadotril

Rececadotril bears the chemical name (RS)-Benzyl 2- ({2-[(acetylsulfanyl)methyl]-3 -phenylpropanoyl} amino}acetate (IUPAC) (empirical formula: $C_{21}H_{23}NO_4S$; MW: 385.48) and different commercial names in different countries for example, Dirasec, Aquasec in India; Cadotril in Peru; Tiorfanor, Tiorfast in France and so on.

ThoughORS has been recommended by WHO as the sole treatment of diarrhea, except the administration of antibiotic in case of severe diarrhea(the predominant symptom of cholera).Few studies have demonstrated certain small molecules to be effective against treating diarrhea and Rececadotril is one of them. This lipophilic antisecretory drug molecule being a neurotransmitter, acts as inhibitor of enkephalinase which degrades enkephalin, a pentapeptide present in brain, GI tract and other parts, thereby helping in gastro intestinal motility, respiration, controlling the cAMP level in the intestinal cells.

The action mechanism of Rececadotril commences with rapid hydrolysis of the drug upon oral administration into thiorphan, which is a more potent inhibitor of enkephalinase, a cell membrane peptidase. Rececadotril gets absorbed quickly upon oral administration and the inhibiting activity of plasma enkephalinase starts with in 30 minutes. Inhibition of this enzyme renders enkephalin protected from degradation and able to bind to its Opioid receptor on intestinal lining cells which in turn reduces cAMP production by inactivating G protein and thus reduces water and electrolyte secretion from the intestine (www.patient.co.uk, 2013). Upon oral administration, radiolabelled study showed no invasion of rececadotril in brain and doesn't affect the gastrointestinal motility (Schwartz, 2000). Clearance of the drug occurs via urinary tract.

A double blind and placebo study performed by Jean and colleagues (Jean *et al.,* 2011) has suggesting the use to Rececadotril as an adjuvant treatment along with ORS in the children in case of acute diarrhea has showed significant lower volume of stool within first 48 hours when administered orally thrice at a concentration of 1.5mg/kg, similar result has been observed while studying the effect of Rececadotril against *E. coli* ,*Shighella* infections (Hamza *et al.,* 1999). Another study has also demonstrated a reduction in stool volume in children by around 46% along with reduction in the intake of ORS in children as well as in adults (Eduardo*et al.,* 2000). A 10mg/kg Rececadotril oral administration study in dogs has also demonstrated the reduction in secretion of water and electrolytes due to cholera toxin, though it couldn't show any change in the basal adsorption rate (Primi *et al.,* 1999). Rececadotril has shown better resolving efficiency, tolerability, and lesser side effects(like rebound constipation and abdominal pain when compared to loperamide (another effective drug against diarrihea) (Vetel *et al.,* 1999; Prado 2002; Hwang *et al.,* 2005).

In spite of having profound beneficial effect Rececadotril is yet to be considered as a first line treatment of cholera as it does not provide any additional benefit in the adult patient with severe cholera as found in a study in Bangladesh where rececadotril effects were compared with placebo patient group where it shows little or no better result than placebo studies (Alam *et al.,*2003) and also causes side effects like dizziness, malaise, headache, and

hyperkalemia in children with severe diarrhea. Besides these there are several conditions for the administration of rececadotril such as; the sugar content of the formulation of the drug has to be considered in case of diabetes, in case of acute dysentery with presence of blood will limit the use of the drug.

3.4 Vaccination

The formulation of cholera vaccine started around a century ago by a Russian Bacteriologist Waldemar Haffkine (1860-1930) by developing an attenuated form of the bacterium. In 1892, Haffkine tested the vaccine on himself and reported the findings and afterwards he moved to India during an ongoing epidemic.Since then, number of notable approaches has been made to develop an ideal cholera vaccine with promising outcomes.

The earlier approaches to formulate the vaccine was to use acellular or phenol inactivated whole cell which provided protection only for a short duration and also showed reactigenicity and hence it was necessary to develop a better vaccine for the bacterium. These limitations lead to the production of vaccines with newer approaches (Joachim and Karl, 2002).There are several biological concerns have to be taken into account while developing the cholera vaccine for example, removal the appropriate virulence factor, incorporation of the elements needed for infection without bringing about the lethal symptoms and so on.

It was assumed that the live attenuated and removal of Cholera toxin gene ($ctxA_1$) will be an ideal candidate for vaccine as CT is the actual component of the bacterium that leads to the symptoms, (Kaper *et al.,* 1984) but even this approach showed symptoms to some extent to prove that there are other virulence factors present for example, Hemagglutinin protease.

There are several new developments and administration studies made in past 2-3 decades demonstrating some extent of efficiency but not without limitations. A double blind field trial in Bangladesh using only whole killed cell as well as B subunit killed whole cell showed lower efficacy in children (23% and 26% respectively) and lower protective efficacy compared to older approach. Older patients showed protection even in third year while it was absent in children. This study also showed better efficiency against classical strain than E1Tor upon multiple administrations (Clemens *et al.,* 1990). Similar study showed 64% protection when applied in combination in endemic areas of Bangladesh (Black *et al.,* 1987) and in South America (Sanchez et.al., 1994). B subunit whole cell vaccine showed the similar result with higher titer of IgA when administered orally (Svennerholm *et al.,* 1983). On the other hand, E1Tor strains from Peru and Bangladesh were attenuated by deletion of the virulence genes and RS1.The ctxB was reintroduced in the genome and this vaccine showed effective outcomes with least reactigenicity (Taylor *et al.,* 1994). Another novel approach was taken by formulation of strain CVD110, a ctxA deleted vaccine E1Tor strain which also lacks other virulence factors for example, zot, responsible for affecting cell junction integrity, Ace (accessory cholera enterotoxin) and hemolysin. This proved to be a potential immunogen with a limitation of showing symptom (Tacket *et al.,* 1993). In 1999,a new CTX negative hemaglutinin/protease defective strain was formulated for E1Tor ogawa which showed significant serum concentration of anti ogawa IgA with a little adverse effect of diarrhea (Benitez *et al.,*1999).In Peru, a live-attenuated O1 vaccine candidate has been formulated which is active against E1Tor Inaba. At a dose of approximately 10^8 CFU it is effective in adults (USA) as well as in children and infants [Bangladesh] while excretion was higher in the USA patients (Chowdhury *et al.,* 2009). In the slum area of Kolkata, India, first long term efficacy of two-dose bivalent whole cell killed vaccine have been demonstrated in a double blind, placebo controlled trial, where around 65% of protective efficacy was

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observed at 5 years in non pregnant patients (>1 year) suggesting its candidature of potent and rational vaccination in endemic settings (Bhattacharya *et al.,* 2013).

A widely studied live oral cholera vaccine strain is O1-CVD 103-HgR, characterized by deleted ctxA and harboring a gene encoding Hg^{++} resistance, which is a significant advance in the research of cholera vaccine. This strain was found to be well tolerated and highly immunogenic in both adults and children upon administration of a single dose (Levine and Kaper,1993; Suharyono *et al.,* 1992). The same vaccine made with classical inaba O1569B showed high protective efficacy against induced moderate and severe cholera caused by E1tor in US study population in a double blind, placebo controlled study (Tacket *et al.,*1999). In two different studies in Indonesia and Thailand CVD 103-HgR showed higher efficiency in 5-9 year old children administrated with the vaccine in different concentration while single dose $5x10^9$ CFU dose proved to be a highly efficient treatment and can be considered of further advancement (Arehawaratana *et al.,* 1992). Besides these studies, the efficiency has also been proved to be dependent on the study area, as this vaccine produces lower vibriocidal antibody titer in patients (common in children) belonging to developing or underdeveloped countries which might be caused by the proximal small bowel bacterial overgrowth diminishing the effect of the vaccine (Lagos *et al.,*1999). A comparison between O1 CVD 103-HgR and WB-rBS has shown high efficiency by both the vaccines while former showed 60-100% protection and WC-rBS showed around 80% protective efficiency for at least six months (Ryan and Calderwood, 2000). This particular strategy was also found to be tolerated in Peruvian adults and seroconversion efficiency was slightly higher in the High Socio-economic levels (Gotuzzo *et al.,* 1993).

Attempt to make attenuated vaccine against O139 strain has also been made by deleting the multiple copies of CT genetic element and reincorporating only B subunit in two virulent strains MO10 and A14456, giving rise to two strains Bengal 3 (which further modified stable, spontaneous, non motile Bengal-15) and VRL-16.Among these three Bengal-15 was found to be safe live attenuated effective candidate vaccine for treating cholera cased by O139 strains (Killeen *et al.,* 1995).

Besides these well characterized and efficient vaccines, there are few other vaccine formulations which have shown significant adverse effects and hence, were not taken forward like, formalin toxoid: reasonably immunogenic but can be reversed to toxic, Glutaraldehyde toxoid: stable but poorly immunogenic (Holmgren *et al.,* 1977) and so on. To make the cholera vaccine affordable in all the countries attempts have been made to formulate inexpensive vaccines such as the whole killed cell vaccine produced in Vietnam had been tested and patients obtained two dose vaccine showed around 66% protective efficacy in Hue (Trach *et al.,*1997), similarly, the same vaccine but modified ,showed significantly lower cholera episodes compared to placebo group in 1-5 years old children at Kolkata, India without any serious adverse effects (Sur *et al.,*2009).

The current status of vaccine usage prescribed by WHO recommends the use only in complicated emergency conditions, decision making tool have been improvised for administration of the vaccines which includes:

a) Risk assessment for an outbreak.
b) Analysis the capacity to contain the outbreak.
c) Feasibility of the OCV campaign in the outbreak area.

Due to the unsatisfactory efficacy and severe adverse reactions, the parenteral vaccine was never recommended. Presently only two oral vaccines are internationally licensed, one consists of Whole Cell/recombinant B Subunit which can be administered in two doses with an interval of 10-14 days. This vaccine needs to be administered in large volume of liquid

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which cannot be given to children of less than 2years off age. CVD 103-HgR is live, attenuated, genetically modified and the second licensed vaccine which shows about 95% protection but is not recommended due to its complicated interpretation and failure to demonstrate convincing efficacy. Hence, the production of this vaccine is stopped since 2004 (Chaignat and Monti, 2007). A report by WHO in 2010 depicts the recent available vaccines which are i) Dukoral and ii) Sanchol and mORCVAX which is compared in Table 4.

Though vaccination is making rapid advancement in the treatment studies of cholera, still it has some limitations to be used as sole treatment strategy. Though extensive research works are going on to overcome the disadvantages such as production of single dose vaccine instead of double dose vaccines, which is less affordable once an outbreak starts still several other limitations like possessing significantly different immune response in middle and lower income countries requiring more intensive research on it (Desai, *et al.,* 2014), the maximum efficacy is yet to be obtained in any kind of cholera vaccine eliminating the adverse effects of abdominal discomforts, diarrhea,by considering the chance of toxicogenic reversion of vaccine cells if not inactivated or killed properly. An efficient vaccine for the children of <1 year of age is yet to be formulated. Even if these limitations are addressed, there are other considerations such as its availability and cost effectiveness in the developing and even in developed countries, for which there are needs of evaluating proper efficacy of a particular vaccine so that the cost to benefit ratio can be determined (Clemens *et al.,* 1996), and the limitation of safe drinking water to be used for vaccination in a cholera hit area.

Properties	Dukoral	Sanchol and mORCVAX
Country of Development	Sweden (1991)	Vietnam, with the name ORCVAX (1997), reformulated in 2004 as mORCVAX.
License year	1991 as Dukoral (not licensed for children less than 2 years).	2009 in Vietnam as mORCVAX; Sanchol in India.
Strains used	Classical, E1 tor, Inaba, Ogawa.	O1 and O139
Formulation	WC-rBS: Monovalent vaccine , heat killed V.cholerae O1, recombinant B subunit of CT toxin.	BivWC: Bivalent vaccine with whole cell.
Shelf life	3 years (2-8^{o}C) ; 1 month (37^{o}C)	2 years (2-8^{o}C)
Dosage	>5 years: 2 oral doses ≥7 days to <6 weeks apart. 2-5 years: 3 oral doses ≥7 days to <6 weeks apart.	≥1 year: 2 oral doses 14 days apart. Booster dose recommended after 2 years.
Adverse effects	Abdominal pain, diarrhea.	
Immunogenicity	Antibacterial +antitoxin antibodies IgA.	Antibacterial.
Efficacy	Among children classical and E1Tor combined protection was 100%. Decreased to 38% and 47% after 1st and 2nd year. 0% thereafter. In vaccines >5 years the rate was 78% in 1st and 63% in 2nd year.	Primarily it showed less effective short term protection than Dukoral but further modification gave a protective efficacy of around 66% in all age groups.
Cost *	60 $ per pill + 10$ shipping charges (different companies).	US$ 1.00 per dose + shipping charges.

Table 4: Comparison between the two most widely used vaccination of cholera.
*Extracted from www.pharmacychecker.com (Dukoral pricing comparisons).

In addition, till date, several vaccination strategies are less than ideal compared to the requirements of epidemic settings in underdeveloped countries without proper

storage facilities, continuous movement of population and other important factors. Moreover, mass vaccination requires advance planning, not only from a clinical or medical point of view but also critically considering socio-demographic, cultural aspects as proposed in a study regarding vaccination acceptance study in Zanzibar (Christian, *et al.,* 2013), which is probably possible for an endemic where onset of disease has an identifiable pattern but much less likely for an outbreak.

3.5 Homeopathy

Though the mechanism of action is yet to be understood since the earliest time of treatment of epidemics including cholera, homeopathy treatment has proved its dramatic efficiency in number of cases masking any other conventional and reliable treatments such as antimicrobial therapy to a certain extent, oral rehydration therapy and so on. The examples and outcomes of homeopathic treatment for cholera and few other epidemics published till date can probably give it the crown of most effective treatment so far. Few instances to mention regarding these facts, back in 1800s, during a cholera outbreak in Europe, came from the east, a hospital practicing orthodox medicine in Vienna reported a cure rate of 30% where as under homeopathic treatment the rate was found to be 67% , the similar results were reported in other places of Europe as well (Robert 2013), at the same time in several other places reported the comparable results for example, London (9% mortality), Bavaria (7%), Austria (33% with an allopathic rate of 66%). Similarly in Russia during 1830-31 homeopathy showed a higher curing rate. In, 1854 at Palermo, an incidence of cholera allopathic treatment showed a mortality rate of 42% where the same for homeopathic treatment was only 4%. In the same year, a cholera outbreak in London showed only 16% mortality under homeopathic medication against 52% of orthodox treatment including antibiotics (Tolpežņikovs, 2012). In 1849, in USA, patients under allopathic and other treatment shoed a mortality rate of 40-70% where as the mortality rate of cholera patients under homeopathic supervision was significantly low i.e. 3% , most importantly, without any side effects (Ton, 2009). Another randomized study at Nicaragua in 1991 showed decrease in number of deformed stool by 4-5 days under homeopathic medicines like, podophyllum, chamomilla, arsenica album, sulphur while treating childhood diarrhea (Jennifer *et al.,* 1994). Benjamin Joslin, in his book entitled Homoeopathic treatment of epidemic cholera, one of the most famous books written in this regard has depicted more pictures on this issue, such as in Vienna, homeopathy showed 8% mortality compared to 31% of allopathic and many more, as well as the comparison between orthodox and alternative treatments including the significant failure of orthodox treatment in number of cases. Joslin, in his book has also suggested the preventive measure for cholera by two medicines i.e. Veratum album and Cuprum metallicum as well as few others like, rhus radicans, chlamolla, stramonium with proper attenuation (diluting to such a point where the solution is nothing but the solvent) which can prevent the cholera when prepared and administered according to homeopathic method. In certain cases camphor was also an effective option though it might have the side effect of interfering with other medications (Joslin 1854).

In spite of being such an effective and inexpensive treatment measure for cholera and several other epidemics, homeopathic medication is yet to get a status of recommended treatment strategy. The awareness of efficacy of this is low in western states in spite of number of positive observation. There are other controversies like ‘anti homeopathy lobby’ of WHO as mentioned by Harry Van Der Zee, MD editor of International Journal For Classical Homeopathy in his letter to WHO where he has depicted the anti homeopathy lobby by Young Scientists in UK supported

by pharmaceutical companies, which has opposed both research and application of homeopathic treatments
(Harry 2009). Other considerable factors in an epidemic setting might be the specification of medicines for each patient undergoing homeopathic treatment as it takes into account not only the patient's physiological but also the psychological as well as emotional status for optimal results. But, considering the history of homeopathy treatments, it is quite justified to hope for the survival and development of this particular benevolent yet effective treatment.

3.6 Zinc supplementation

The relation between intestinal disorders and Zinc was first established by Kelly *et al.,* during studies of acrodermatitis enteropathica in 1976 (Tomkin *et al.,* 1993) suggesting the onset of persistent diarrhea due to slow parasitic excretion in zinc deficient conditions which is quiet prevalent in developing countries. Since then, like other treatment measures zinc treatment is also studied to combat diarrhea and thus cholera. The documented mechanism of zinc is to restore the integrity of mucosal barrier which can be detected by lower lactulose excretion (Roy *et al.,* 1992), it also blocks the potassium channels (Lazzerini, 2008; Hoque and Binder, 2006) , hence indirectly resisting the chloride loss and ion secretion due to cAMP occurred by cholera toxin [but not effective against E. coli heat stable enterotoxin induced ion secretion (Canani *et al.,* 2005)] and finally, zinc when administered in the form of acetate or sulfate, also increases the antibacterial antibody titer. The effect of zinc on antibody production has also been supported by another study in Bangladesh, especially when co-administered in 20 mg amount with WC-rBS vaccine along with temporary withhold of breast-feeding in <2 years aged children (Ahmed *et al.,*2009; Karlsen *et al.,*2003; #Ahmed *et al.*,2009; Albert *et al.,*2003). Interestingly, under same condition zinc was found to increase the titer of vibriocidal antibody and significantly less production of CT-antibody suggesting its different modulatory effects for these antibodies (Qadri *et al.,* 2004).

Over last 2-3 decades extensive research and field trials have been performed in developing countries like Bangladesh,and India to evaluate the efficiency of the agent to treat diarrhea as well as cholera. A number of double blind, placebo controlled studies in Bangladesh have supported the beneficial effect of zinc in children of different age groups where the patients supplemented with zinc have showed significantly lower stool output and lesser diarrhea duration depending on the dose, for example, 20mg elemental zinc /day reduced 28-39% stool weight compared to control (Khatun *et al.,*2007; Roy *et al.,*1997), also reported to reduce diarrhea risk by 23% in New Delhi, India (Sazawat*et al.,* 1995), 4-5mg/Kg BW/ Day was also reported to be beneficial in <3 years old children (Hidayat *et al.,* 1998). Similar results were obtained at same concentration when applied on malnourished children (Polat *et al.,*2003) where as 30mg Zn/day could reduce duration of diarrhea by 12% and only 11% stool output in comparison with control (Roy *et al.,* 2007). Another study was performed to detect the efficacy and difference in efficacy for Zn supplement between two different administration strategies i.e. 10mg for 5 days/week and single dose 50mg/week, both the doses showed almost 50% lesser number of children suffering from diarrhea compared to control (Gupta *et al.,*2007), duration of diarrhea also showed a decrease when Zinc was administered in 14mg/day for 15 days in <2 years patients (Faruque *et al.,* 1997) , was also found operationally feasible (Gupta et.al,2007). Zinc treatment was found to decrease diarrhea mortality and episodes as well as risk of hospitalization (Walker and Black' 2010; Bhatnagar *et al.,* 2004). The supplementation of Zinc was reported to be correlated to weight gain in

children while suffering from diarrhea (Sur *et al.,* 2003) and the treatment was not found to be interfering with the conventional remedy of ORS (Awasthi, 2006). Another recent study in rats has demonstrated the correlation between the Zn deficiency and the production of nitric oxide which facilitates the cellular damage in intestinal cells. Zn supplementation can reverse it by causing expansion of lamina propia, constituent of moist lining of GI tract, implying better absorption and decreasing NF-kB – DNA binding indicating lesser amount of cell death as well (Altaf *et al.,* 2013). In addition to the Zn, most of the studies also recruited Vitamin A, but the efficiencies were found to be insignificant.

On the contradiction, studies have shown adverse effects of Zinc supplement such as frequent episodes of vomiting in supplemented children than control placebo group (Strand *et al.,* 2002) , reduction in the plasma copper levels as well as no effect on weight gain in malnourished 06-36 months aged children in Pakistan (Bhutta *et al.,*1999) and so on. Similar inefficiency was reported in children of <6 months age taking zinc acetate 20mg/Day and elemental Zinc 5mg/Day (Brooks *et al.,* 2005). Another trial in infants in India ,Pakistan and Ethiopia documented increased diarrheal episodes with Zn supplementation and no difference in vomiting and stool frequency in supplemented and control groups suggesting zinc supplementation treatment to be ineffective in the treatment of diarrhea and hence cholera in infants (Walker *et al.,* 2006).

Even though, efficacy of Zn treatment in diarrhea has been well established by various studies, still , all these adverse observations has to be considered while recommending the Zn supplementation as a first line treatment strategy for diarrhea in cholera. Along with these, an optimal dosage has to be determined and extensive studies to be performed to determine the effect of Zn absorption on iron and calcium absorption as a competition between these micronutrients may lead to undesired negative effects (Khan and Sellen, 2011), besides, proper dosage for the bio-availability of zinc itself has to be determined individually for different age groups and considering other health factors such as nutrition status, micronutrient status, and so on., otherwise it has been reported to cause severe adverse effects for example, a dose of 300mg/day in healthy individuals (70kgBW) have shown interrupted polymorphonuclear and lymphocyte functions by reducing lymphocyte proliferation in turn hampering immune responses, which is very near to the dose of 20mg/day in a 6 or 7 years aged child (Chandra, 1984). Hence it is of high importance to optimize the efficacy of this strategy in different geographic populations such low, middle and high income countries as well as in normal and malnourished infants and children.

3.7 Bacteriophage based biocontrol

Bacteriophage is virus that infect bacteria and replicates inside either as a separate entity inside the bacterial cell or integrating its genetic material into bacterial chromosome, finally comes out by bursting the bacterial cell (lytic) or stays in the host cell as temperate (lysogenic). The phage having the capability of killing the bacteria is the lytic phage as its bursts open the cell. Hence, lytic phages are of immense importance as far as bacterial disease control is concerned.

The treatment of bacterial diseases using bacteriophage has both advantages and disadvantages. Few of the advantages are the specificity of the phage for a particular bacteria hence it does not raise the risk of killing of other beneficial bacteria which is a main drawback of the antibiotics. The other advantages are the no major side effect of phage treatment, easy administration. The most important advantage of the phage therapy is the resistance of the bacteria for its particular phage which is again, one of the main concern regarding antibiotic therapy, the

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receptor for the phage on the bacteria is also the virulence factor, hence; if the receptor gets mutated then certainly its virulence for human will decrease which is of course a positive bottom-line. Production is also simple, less time consuming and less expensive compared to the antibiotics. In spite of having such enormous advantage phages are also not without flaws, the high specificity of phages will require extensive search and identification of the particular phage for a particular bacterium. The detailed knowledge of the bacteria is needed for characterization the receptor, the gastric acidity also has to be neutralized to protect the phage proteins from the low acidity , the infecting phage has to be lytic phage to get desired result (Sandeep K., 2006).

The account of bacteriophage research as a promising measure for cholera can be distinctly divided into a couple of periods, starting from early 2000 century. Twort and d'HERELLE are the names mostly pronounced regarding the discovery of bacteriophage, though it was Ernest Hankin in 1896 to observe the agent, then unknown bacteriophage, with the vibriocidal capacity in Ganga-Junma river water but without any further investigation (Deresinski, 2009). It took another couple of decades to observe the same by Frederisk Twort in 1915 and Felix d'Herelle in 1917 at Pasteur Institute during World War I (Monk *et al.,* 2010). It was d'Herelle indeed; who took the phenomenon seriously and took it forward with the impression of its efficacy to treat bacteria caused epidemics. Since then it was quite a struggling journey, especially in India, suffering then from cholera epidemic, for d'Herelle to establish his discovery. William C Summers, in his article entitled 'Cholera and Plague in India: The bacteriophage Inquiry of 1927-1936', gave an excellent account of the period of d'Herelle's discovery, establishment of his notion that the phage is a virus and the struggle to experiment on the phage therapy to treat plague and mainly cholera in India in an extremely complex religious, political and social environment as that particular period was the period of Indian Independence Movement and a time of Non-cooperation with the British Government. Still, with all the effort of the Government, Indian Medical Service (IMS), IRFA etc it was possible to carry out several trials to determine the efficacy of phage treatment. There are a number of names for example, Dr M. N. Lahiri, Dr. Pasricha, Dr. Asheshov and many others along with their experimental efforts to mention in order to complete the description of the era which ended approximately in 1940s with no impressive outcome of the therapy, though the experiments in Assam and few other provinces showed extraordinarily low mortality rate. The most probable reasons of the trials not found to be impressive and conclusive enough were the complex situation of the country, inefficient experiment design, untrained personnel, financial limitations. The chapter of bacteriophage was considered to be closed with the arrest and execution of Eliava by Stalin (Chanishvili *et al.,* 2001), the co-founder of Eliava Institute of Bacteriophage in Georgia, along with d'Herelle, one of the most important institutes to carry on bacteriophage research, once the political disturbances appeared due to the World War II (Summers, 1993). The incident made d'HERELLE frustrated and disillusioned and compelled him to leave Georgia (Sulakvelidze *et al.,* 2001).

The next few decades the West countries attained a dormant stage in bacteriophage research as they were immersed in antibiotic studies. Asiatic countries including USSR (Soviet Union) though still continued their research in bacteriophage therapy having contradictory outcomes of both positive and negative observations for cholera. To mention a few, a study in 1971 with cholera patients treated orally and intramuscularly with bacteriophage showed lesser efficiency than tetracycline antibiotic (Marcuk *et al.,* 1971) whereas

bacteriophage efficacy in treating diarrhea in rabbits (Dutta *et al.,* 1963) and in human phage preparations containing $2x10^{12}$ pfu/ml (Monsur *et al.,* 1970) showedhopeful outcomes. A different than conventional preparation of phage i.e. cultivating vibrios through alternate passages in guinea pig intestines and bile when administered with cholera vaccine showed no further incidence of cholera in Afghanistan (Sayamov, 1963). In addition to human administration, phage therapy is already in use in the food industries to make the food materials safe from several bacterial infections. No negative effects on the efficacy of other drugs due to bacteriophage use has been found though systemic studies are yet to be carried out in this regard (Kutter *et al.,* 2012).

A very recent study based of difference of phage, ORS and antibiotic treatment has been performed where ciprofloxacin showed a better combating capacity compared to ORS and phage both but from a specificity point of view phage treatment was promoted by the investigators (Jaiswal *et al.,* 2014). Another study demonstrated the effectiveness of phage cocktail in rabbit model was more if administered after the bacterial contamination or infection rather than administering as a precaution (Jaiswal *et al.,* 2013). In addition to this several other trials are going on in order to determine the phage efficacy and its validation.

Along with the clinical experiments, numbers of review articles have been published in last few decades to discuss the status as well as the considerations while experimenting and further aspects of phage therapy. The main concerns to be address were found to be the specificity of phage for each and every strain of bacteria because phages are certain times strain specific even, antibody production against the phage particles which in turn can render them inactive (Inal 2003; Mathur *et al.,* 2003) and the concern for the rapid uptake of phage particles by spleen. Merril *et al.,* have suggested the need to determine the efficacy and pharmacokinetics for phage in treating bacterial diseases such as cholera (Merril *et al.,* 2003). There is another important factor to be noticed is the preparation of the phage as an optimum concentration of the phage solution is required to get the most effective results and this issue has been also addressed in reviews (Jason and Paul, 2010). A handsome account regarding the state of the art approaches to utilize the phage in therapy has also been reviewed by Kutter and colleagues (Kutter *et al.,* 2010).

There are several other questions besides the already mentioned, to be answered by the researcher community regarding the safe and efficient transportation to the epidemic areas, optimum preparation technique for the phage solution, optimum dosage of the same as well as the concern of long term efficacy in order to establish the phage therapy as a well recommended and effectively used cholera treatment strategy.

3.8 Toxtazin

The limitations of the existing cholera treatment strategies, mostly the emergence of antibiotic resistant *V.cholerae* strains, have leaded the researchers towards several directions like identification of the small bacterio-toxic moleculesfor example, Toxtazin. After Virstatin found to be a ToxT transcriptional regulator inhibitor which works by inhibiting the dimerization of the mentioned transcriptional activator of virulence genes for example, CT, Tcp (Shakhnovich *et al.,* 2007), one more small molecule with the capability to affect the ToxT have been identified called Toxtazin (Rebecca and Victor, 2013). Both the CT and Tcp genes are transcribed under the influence of ToxT, a transcription factor. The researchers identified three molecules of Toxtazin class and named Toxtazin A, B and B′. All the three compounds could reduce the production of both CT and Tcp, Toxtazin A was found to act at the toxT promoter without any effect on bacterial colonization capability while the other two

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structural analogs act at tcp promoter, probably at tcpPH, the factors essential for toxT transcription, reducing the colonization by 100 fold (Rebecca and Victor, 2013). Hence; the study strongly supports the capability of these small molecules to become an efficient treatment, though the strategy is still in its infancy and needs further extensive research along with clinical and field trials in order to get validation.

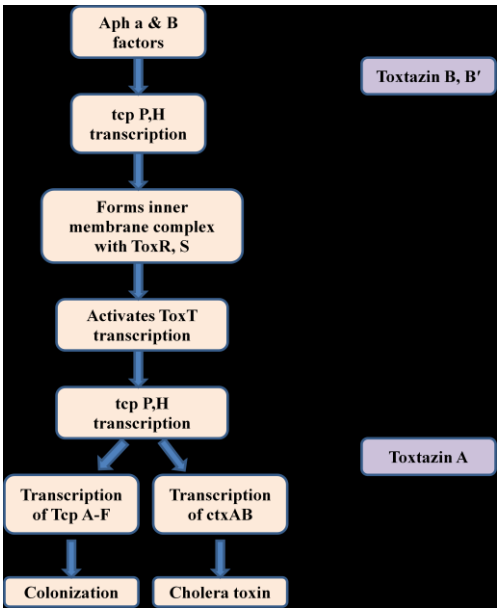

Figure 3: Mechanism of action for the small molecules (Tox A, ToxB and Tox B′)

3.9 Other strategies

There are few other isolated treatments strategies have been formulated and studied such as deactivation of the cholera toxin by using sialidase or weak acids against sialidase sensitive di or tri monosialosylganglioside which renders them capable of toxin inactivation and thus can be a potent strategy (Carolyn and William, 1973). Another very tricky approach was to mimic the enterocytes receptor of cholera toxin on harmless gut bacteria capable of adsorbing toxin more that 5% of its own weight and was found to be effective (Antonio*et al.*,2006). Though these approaches found to be useful, still has to go through more extensive research and clinical studies.

4. Conclusion

The overview of the treatment measures for cholera and related water-food borne diseases gives a basic idea of the advantages as well as limitations. Where all the strategies are dedicated towards curing cholera or other diarrheal diseases, it is far more beneficial to prevent this and thus preventing the deaths. Approximately 17% of the world population is without improved water and even more do not have the access to the safe drinking water (Moe, 2009). Diarrheal diseases kill around 4000 children less than five years of age per day all over the world even after rapidly growing treatment methods, mostly in the developing countries (GOV.UK policy, 2013). Hence; the first and foremost as well as most beneficial preventive measure would be to provide safe and clean drinking water free from any chemical or bacterial contamination. There are several initiatives taken in both the developed and developing countries to address these issues such as the 'Providing clean water and sanitation in developing countries' policy recently published by Government of UK (GOV.UK

Policy, 2013) , potable water privatization programs ,improvement of population's access to safe drinking water and sanitation services in Latin America (Moe 2009) and more. These policies are certainly one of the best preventive measures against the water borne diseases such as cholera, but the implementations of these policies are even time consuming.

In addition to these mentioned preventive measures there are other techniques to get rid of this such as physical removal of the pathogens by filtration or sedimentation, chemical treatments, heat and UV treatment etc (Thomas and Sandy, 2004; Gadgil, 1998), In fact simple solar treatment has also shown beneficial effect on preventing diarrheal diseases in children under 6 years of age in Kenya (Conroy *et al.,* 2001). In 1999, in Madagascar, Cooperative for Assistance and Relief Everywhere (CARE) and Centers for Disease Control and Prevention (CDC) took an initiative in safety of drinking water by marketing a sodium hypochlorite solution named Sûr'Eau and was applied till 2001 in Fort-Dauphin, Madagascar to treat cholera and was found protective against the diseases (Meganet*et al.,* 2001).

In brief, the in practice treatments and the policies regarding safe drinking water supply and developed sanitary services are no doubt the most promising curing measures available so far but still, it would be more beneficial to prevent the occurrences and spread of cholera and for which intense water treatment researches are needed to be carried out. The treatments such as vaccination and bacteriophage methods might be the best beneficial methods but must be subjected to the availability and cost effectiveness concerns for the end users who are not even able to pay for drinking water. Treatment by homeopathic treatments showed a promising outcome but unfortunately was disregarded in this context, which should again come into action in the form of research and clinical trials as bacteriophage did after the emergence of antibiotic resistant bacterial strains. The main objective of over viewing the methods is to intimate the severe disadvantages experimentally observed of the already existing methods which are actually overshadowing the efficacies of the same in respect of efficiency, cost effectiveness, availability in an epidemic settings and so on.and to promote the need of drinking water treatment, in general or at point-of-use might be in the form of strong waste water treatment policies (Okun, 2000) or by formulating stronger, more Universal, well studied and characterized solutions like Sûr'Eau which will be more inexpensive, easily accessible and can be used by the end user directly to treat any kind of water they can access in most of the developing countries who are, in certain countries for example, South Africa, not even getting benefit of Government privatization of drinking water, as such noble policies are also compelling them to procure their water from polluted lakes and rivers due to their inability to pay for water (Jacques 2003). Even still, these methods may have to overcome the socio-cultural or baseless belief barriers of people such as in Zimbabwe for instance, where people belief water is always pure and should not be supplemented with anything (Knapp and Ogunbanjo 2008), which can be achieved by awareness and education programmes. But it is high time to put emphasis on consideration of diverging the main stream treatment and cure oriented research into preventive measures as well, by novel strategies not only in supply level but also at individual consumption level. Also the application of mathematical models regarding epidemic and endemic dynamics or transmission and spread of cholera considering the contaminated water sources (Wang and Liao 2012; Shuai and Driesseche 2014) in complementation of the above mentioned preventive strategies will certainly facilitate the cause under any parameter and settings.

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