Herbal Compounds-An Alternative for Multi-Drug Resistant Vibrio Cholerae

Sabah Perveen and Hotam Singh Chaudhary

Department of Biotechnology, Madhav Institute of Technology and Science-Gwalior, RGPV, India

Abstract: Vibrio cholerae is a causative agent of cholera, many people dies every year, especially in developing countries around the world. The outbreaks of cholera are responsible for approximately 120,000 deaths annually. Cholera is a self limiting illness; however antibiotics are used as a part of treatment regimen. At present, the treatment against cholera has become very critical issue worldwide, because most of the strain developed multidrug resistance. Efflux pumps, spontaneous chromosomal mutation, conjugative plasmids, SXT elements and integrons are discussed as an antibiotics resistant mechanism. Now at present the demand is to find an alternative and promising strategy and development of novel therapeutics. The present chapter is mainly focus on the treatment, strategies and developing resistance against these antibiotics. Later section mainly focused on the utility of natural remedies against V. cholerae infection.

Keywords: Antibiotic Resistant, Medicinal Plants, SXT Elements, Photochemical, ToxT, Vibrio Cholerae

Introduction

V. cholerae, a member of the family Vibrionaceae is a facultative anaerobic, Gram-negative, non-spore-forming curved rod, about 1.4-2.6 mm long, capable of respiratory and fermentative metabolism; it is well defined on the basis of biochemical tests and DNA homology studies (Baumann et al., 1984). Discovery of V. Cholerae is credited to Filipo Pacini, who first time describes V. cholerae and also made microscopic slide first time. V. Cholerae is classified by the heat-stable surface somatic “O” antigen, present in the outer polysaccharide layer. This classification was firstly described by Gardner and Venkatraman (1935). Presently the organism is classified into 206 “O” serogroups (Shimada et al., 1994; Yamai et al., 1997). Until recently, epidemic cholera was exclusively associated with V. Cholerae strains of the O1 and O139 serogroups. The O1 serogroup exists as two biotypes, classical and El Tor; antigenic factors allow further differentiation into two major serotypes- Ogawa and Inaba. The cholera was originated in the India subcontinent, it has been prevalent in the Ganga Delta from ancient times (Sack et al., 2004). The first cholera pandemic occurred in India in Bengal region starting in 1817 through 1824. The second pandemic lasted from 1827 to 1835 and the affected countries were United States and Europe due to the results of advancement in transportation and global trade and increase human migration. The third pandemic began in 1839 and persisted until 1856, extended from North America to south America for the first time specially Brazil. During fourth pandemic from 1863 to 1875 cholera hit the sub-Saharan African region. During 1881-1896 and 1899-1923 the fifth and sixth pandemic occurred. Seventh pandemic originated in 1961 in Indonesia and is marked by the emergence of new strain named E1 Tor which still persistent in developing countries (Aberth, 2011). The pandemic stages emerged because of the resistance to antibiotic. The strains of V. cholerae became multi-drug antibiotic resistant.

Pathogenicity for Human and Virulence Factor

The genes that enable a strain to infect and cause disease are called virulence genes and the proteins they encode are called virulence factors. Many of the virulence genes in V. Cholerae are located in so-called Pathogenicity islands (PAIs). In V. cholerae these are called ‘V. cholerae Pathogenicity Islands’ (VPI-I and VPI-II) and ‘Vibrio Seventh Pandemic islands’ (VSP-I and VSP-II). These PAIs have been identified by analysis of the G+C content along the genome. The PAIs usually have a lower G+C content than the surrounding DNA. This indicates that these sequences have been
acquired by horizontal gene-transfer, by mechanism similar to those whereby drug-resistance genes are being exchanged. Analysis of the flanking regions indicate that transduction by temperate bacteriophages are a likely source (Dziejman et al., 2002; Karaoilis et al., 1998). In order to cause cholera the strain must carry the temperate phage CTXφ, encoding the Cholera Toxin (CT). The existence of cholera enterotoxin was first given by Robert Koch in 1884 and demonstrated 75 year later by Dutta et al. (1959) working independently. Structural analysis of toxin showed it to consist of a subunit and 5 smaller identical B subunit (Finkelstein and Dutta, 1984 and demonstrated 75 year later by Robert Koch in 1884 and demonstrated 75 year later by Dutta et al. (1959) working independently. Structural analysis of toxin showed it to consist of a subunit and 5 smaller identical B subunit (Finkelstein and LoSpalluto, 1969). The A subunit has a specific enzymatic function and acts intracellularly. It raises the cellular level of cAMP and thereby altering the net absorptive tendency of small intestine to one of net secretion. The B subunit binds the toxin to eukaryotic cell receptor ganglioside GM1. In toxigenic V. cholerae O1 and O139 has a dynamic 4.5kb core region, called as a virulence cassette but not found in non-toxigenic strains (Trucksis et al., 1993). This virulence cassette carry at least six genes including ctxAB (encoding the A and B subunits of CT), zot (encoding zonula occludens toxin (Fasano et al., 1991)), cep (encoding core-encoded pilin (Pearson et al., 1993)), ace (encoding accessory cholera enterotoxin (Trucksis et al., 1993) and orfU (encoding a product of unknown function (Trucksis et al., 1993)). The two major virulence factors in V. cholerae are Cholera Toxin (CT) and Toxin Co-regulated Pili (TCP). TCP is a type IV pilus required for intestinal colonization (Rhine and Taylor, 1994). They cause the bacteria to aggregate in crypts of the small intestine but they not involved in adhesion of epithelial cell. CTXφ phage also gets attached to TCP. The genes for TCP formation (tcpA-F) are located in VPI-1 Pathogenicity Island and the CT genes (ctxAB) are located on CTXφ phage and they both are under control of transcriptional activator ToxT.

**Mechanisms of Antibiotic Resistance**

**Bacterial Efflux Pumps**

Efflux pump used by V. cholerae to export a broad range of antibiotics, detergents, dyes that are structurally and chemically unrelated (Paulsen et al., 1996). VcaM, a V. cholerae ABC (ATP- binding cassette) multidrug resistant pump is a ATP- driven pump. It confers resistant to structurally divergent drugs (e.g., tetracycline, norfloxacin, ciprofloxacin and doxorubicin). V. Cholerae uses an array of MATE (multidrug and toxic compound extrusion)-family efflux systems, namely VcmB, VcmD, VcmH, VcmN, VcmA and VcrM (Begum et al., 2005; Huda et al., 2003). MFS transporters in V. cholerae include the V. cholerae efflux systems (Colmer et al., 1998) that confer resistance to bile (deoxycholate), antibiotics (e.g., chloramphenicol and nalidixic acid) and the proton gradient-uncoupling agent carbonyl cyanide m-chlorophenylhydrazone (Colmer et al., 1998; Woolley et al., 2005). Recently shown that the classical O395 strain carries the MFS efflux protein EmrD-3, which confers resistance to linezolid, rifampicin, erythromycin and chloramphenicol when expressed in a drug-hypersensitive Escherichia coli strain (Smith et al., 2008). Collectively these results show that the efflux pump is exclusively employed in drug resistant.

**Spontaneous Mutations**

Mutation in bacterial chromosomes can also be a reason for antibacterial drug resistant. It has found that mutation cause resistant to the cell wall biosynthesis inhibitor alafosfalin and to the DNA replication inhibitor family of quinolones in V. cholerae (Allen et al., 1979; Gellert et al., 1977; Goss et al., 1965; Sugino et al., 1977). From comprehensive study it was found that
during 1980s 0 cholerla epidemic in the United Republic of Tanzania, the rate of mutation in \textit{V. cholerae} genes is higher than the \textit{E. coli} genes. This facilitates the resistance to antibiotics such as alafosfalin (Atherton \textit{et al.}, 1979). Chromosomal mutation in genes gyrA and parC which encodes subunits of DNA Gyrase and topoisomerase IV, respectively, a resistant against quinolones is developed in \textit{V. cholerae}. There are various reports documented the multi-drug resistance in \textit{V. cholerae} including the antibiotics tetracycline, erythromycin, chloramphenicol, quinolones, streptomycin and cotrimoxazole (Abera \textit{et al.}, 2010; Das \textit{et al.}, 2008; Islam \textit{et al.}, 2009; Karki \textit{et al.}, 2010; Ngandjio \textit{et al.}, 2009; Ranjbar \textit{et al.}, 2010; Roychowdhury \textit{et al.}, 2008; Kumar \textit{et al.}, 2014; 2012).

\textbf{SXT Elements and Integrons}

The SXT elements was first described in \textit{V. cholerae} serogroup O139 based on its ability to harbor genes which provide the host bacterium with resistant to sulfamethoxazole, trimethoprim and streptomycline (Waldor \textit{et al.}, 1996). Beaber \textit{et al.} (2004) has found that the horizontal dissemination of SXT-encoded antibiotic resistant genes is regulated by bacterial SOS response. Further research demonstrated that stress alleviates the SXT-encoded repressor setR, which in turn activates excision and conjugation of the elements. Ciprofloxacin act as an inducing molecule that can promote horizontal transfer of SXT elements. These results suggest that the antimicrobial agents can promote the spread of antibiotic resistant genes. SXT elements have capacity to mobilize conjugative plasmids and genomic islands in trans (Daccord \textit{et al.}, 2010; Hochhut \textit{et al.}, 2000), providing an alternative mechanism for antibiotic resistant gene transfer. All \textit{V. cholerae} isolates facilitates the large chromosomal integrons that provide them the capacity to rapidly transfer gene cassettes containing antibiotic resistant genes (Mazel, 2006).

\textbf{Conjugative Plasmids}

Many \textit{V. cholerae} strains are identified which developed resistant against tetracycline antibiotics, an oral drug often given to patient during rehydration therapy (Greenough \textit{et al.}, 1964). First reported tetracycline resistant strain (exhibiting resistant to tetracycline, streptomycin and chloramphenicol) was isolated in the Astrakhan region of the USSR circa 1970 (Kitaoka \textit{et al.}, 2011). This resistance was transferable to \textit{E.coli} K-12 and thses strains carry a single plasmid. Similarly during cholera outbreak in Bangladesh in 1970 it was caused by the strain which carried a multi drug resistant plasmid transferable through conjugation with other bacteria, including \textit{E.coli} (Glass \textit{et al.}, 1980). This plasmid showed resistance to a no. of antibiotics in addition to tetracycline including ampicillin, kanamycin, streptomycin, gentamicin and trimethopim.

These are the method by which the strains of \textit{V. cholerae} became resistant to antibiotics. Now the main target of present day is to find an alternative for these diseases.

\textbf{Herbal Plants- good antimicrobial Activity: There are many herbal plants present in the nature which has high medicinal plant. These plant can be use as a source of antimicrobial compounds which can be use to kill the Pathogenicity of various pathogenic bacteria. There are various plant reported which showed antimicrobial activity. In historical times, traditional therapeutics used to treat the infection caused by \textit{V. cholerae} from various medicinal plants. The active compounds present in natural compound can be used to treat \textit{V. cholerae} by various pharmacologic mechanisms. Some compound shows direct antimicrobial activity against \textit{V. cholerae} and some inhibit the binding of CT to GM receptors at epithelial cell surface. On this mechanism many herbal compounds identified against \textit{V. cholerae}.

- **Neem:** This is the most important and ancient medicinal plant of India who’s each part has some medicinal value. Neem oil suppresses growth of several species of pathogenic bacteria such as \textit{S. aureus, S. typhosa} (Chaurasia and Jain, 1978) \textit{V. cholerae} (Kunin, 1993)
- **Green Tea:** there are many compounds found in green tea which show antimicrobial property. Extensive research on catechin showed that it inhibits the growth of vibrio cholarae (Borris, 1996).
- **Allium cepa:** Abdul Hannan \textit{et al.} (2010) showed that \textit{Allium cepa} has antimicrobial property and it inhibit the growth of \textit{V. cholerae}. They found that the antimicrobial activity of purple type of allium cepa extract was better as compared to yellow \textit{Allium cepa} extract
- **Indian Species:** Praveen Singh \textit{et al.} (2013), has found that many Indian species showed antimicrobial property against \textit{V. cholerae}. The extract of extracts of Black cardamom (\textit{Amomum subulatum}), Mustard seed (\textit{Brassica nigra}) , Red Chilli (\textit{Capsicum annum}), Bay leaf (\textit{Cinnamomum tamala}), Cinnamon (\textit{Cinnamomum verum}), Coriander seed (\textit{Coriandrum sativum}), Cumin seed (\textit{Cuminum cyminum}), Green cardamom (\textit{Eleetaria cardamomum}), Liquorice (\textit{Glycyrrhiza glabra}), carom seed/Thyme (\textit{Trachyspermum ammi}), Anise (\textit{Pimpinella anisum}), Black pepper (\textit{Piper nigrum}), Fenugreek (\textit{Trigonella foenum-graeceum}), Turmeric (\textit{Curtcuma longa}), Dry ginger (\textit{Zingiber officinale}) showed a significant level of antimicrobial activity against \textit{V. cholerae}.

- **Coccus hirsutus Linn:** Kalirajan \textit{et al.} (2012) studied that the antimicrobial activity of methanol and aqueous extract of herbal plant cocculus...
hirsutus using *E. coli*, *V. cholerae*, staphylococcus aureus, etc. they found that the aqueous and methanol extract of plant is more effective against *V. cholerae* and staphylococcus aureus

- *Psidium guajava*: Also known as “goiabeira” found to have antimicrobial property. Rahim et al. (2010) observed the antimicrobial activity of psidium guajava taking its leaf and bark against *V. cholerae* and suggested that nature of its bioactive component is nonprotiec

- **Garlic Extract**: Researchers found that galactan polyasacharide a bioactive compound present in garlic extract as a major anti-choleric component. Politi et al. (2006) repoted the inhibitory property of galactan against B-subunit of CT

In this context many more researches has been done and reported many neutral compounds against vibrio cholarea. Polyphenol extract of apples shows good anti-choleric properties. They inhibit the enzymatic activity of a subunit of CT (Saito et al., 2002). The therapeutics component present in apple is chlorogenic acid, phloridzin, phloretin, caffeic acid and p-coumaric acid, monomeric Catechins, procynidine. Similar study conducted by Hör et al. (1995) proanthocyanidines extracted from *Guazuma ulmifolia*, a medicinal plant present in Mexico, can provide in vivo inhibitory properties against cholera toxin.

Oi et al. (2002) reported the pharmacological properties of rhubarb galloyl tannin (RG-tannin), an active compound isolated from Rhei rhizome (*Rheum palmatum*), against CT including ADP-ribosylation and fluid accumulation. Studies conducted on animal models (rabbit and mouse) indicated the heterologus polyphenol gallate inhibit fluid accumulation induced by CT.

Chatterjee et al. (2010) reported the bioactive component present in red chilli showed an inhibitory effect against *V. cholerae*. Further study found that the bioactive component capsaiacin present in red chilli act as antimicrobial agent against many pathogenic bacteria *V. cholerae*, bacillus spp. Etc.

Similarly some more compounds found from in-silico and in-vitro studies which are inhibiting the growth of *V. cholerae*. A study in mice reveals that the aqueous and etholic extract of leaves of *spondias mombin* and *Senna occidentalis* and stem sap of *Musa sapientum* against two epidemic strain of *V. cholerae O1* (BA O1 and CVC O1) could be a good alternatives in the treatment of cholera (Shittu et al., 2014). They found in the in vivo studies that the intestinal sample of mice showed mild loss of villi at lower dosages regime and at higher dosages no lesion were observed compare to control groups. This study also suggests that the aqueous extract of *spondias mombin* and *Senna occidentalis* can be an alternative for the treatment of epidemic *Cholerae*.

**Conclusion**

Since antibiotic is widely used for the regime of cholera, the number of pathogenic strain of *V. cholerae* resistant is increasing, as summarized in Table1. *V. cholerae* is an environment organism; it means it has ability to acquire resistance genes from intimate contact with intrinsically resistant environment bacteria (Martínez, 2008) through mobilizable genetic elements. *V. cholerae* can share these resistant genes with other bacteria (Mekalanos et al., 1997; Sedas, 2007) in human gut. To prevent this resistance it is require limiting the use of antibiotics to cholera patient.

The milestone of cholera treatment is to develop a vaccine for the children. Vaccine will prevent the cholera effectively in comparison to other treatment. However, in spite of some recent advances in understanding of host-pathogen interactions, molecular mechanism underlying pathogenesis of *V. cholerae*, still such a vaccine yet not developed (Provenzano et al., 2006).

Another promising approach is to discover the therapeutics by selecting new targets. The drugs that disable the bacterium by inhibiting their virulence mechanism (Hung et al., 2005). Transcriptional activator ToxT, ToxR and TcpP required for the synthesis of cholera toxin and TCP represents a promising target. Because the expression of cholera toxin and TCP is control by transcriptional activator ToxT, if a drug inhibit this target both the virulence factor of *V. cholerae* will not be expressed.

The most promising and significant approach will be the natural compounds. There are many compounds present in the herbal plants which have high medicinal value and these could be used as therapeutics alternatives for these resistant bacteria (Chomnawang et al., 2009).

Recent studies found that there are some herbal plants which have some bioactive compound that can inhibit the growth of *V. cholerae*. Arjun extract of plant *Terminalia arjuna* inhibited that growth of *V. cholerae* (Fakruddin et al., 2011). This study reveals that *Terminalia arjuna* would be a good antibacterial drugs in the treatment of *V. cholerae* infection, provided if found effective and non-toxic through in vivo studies.

Now many of herbal compounds have been found till date, but to find the most potential and drug-like property still remains. From this entire compound to found the most potent compound we can use in-silico method. Computer aided method is an easy and a preliminary steps to screen the novel therapeutics agents and the discipline is an emerging strategy as it reduces many complexities of drug discovery process. By using in-silico method we can found a potential drug for *V. cholerae* and their effectiveness can also be checked by in-vivo studies. This can be a better alternative to found an alternative therapeutics against *V. cholerae* infection. In-silico reduces the complexity and time as compared to traditional method of drug designing.
Table 1. Major drug-resistant *V. Cholerae* strains reported in the last decade

| Year       | Country       | Strain          | Antibiotic resistant                              | Mechanism                                      | References          |
|------------|---------------|-----------------|--------------------------------------------------|------------------------------------------------|---------------------|
| 1993-2005  | Pakistan      | O1 Inaba/Ogawa  | Co (100), Cm (30); Amp, SXT, Cm, Tet             | ND                                             | Jabeen et al. (2008) |
| 1995-2001  | Indonesia     | O1/non-O1       | 1995: Sm; 2000: SXT, Sm; 2000:--                  | ND                                             | Tjamadi et al. (2003) |
| 1995-2000, 2002 | Vietnam     | O1              | Fz, Cpr, Amo, Co Fq (since 2002)                 | ND                                             | Ghara et al. (2004)  |
| Jan 1999-Dec 2007 | India     | El Tor ogawa, O1, O139, non-O1, non-O139 | Fz, Cpr, Amo, Co Fq (since 2002)                 | ND                                             | Chander et al. (2009). |
| 2000-2004  | Hubli, India  | O1              | Co, Sm, Cm, Amp, Tet                             | 26Kb self-transmissible plasmid                 | Chandrasekhar et al. (2008) |
| 2000-2001  | Indonesia     | O1              | Amp, Tet                                         | ND                                             | Rakoto et al. (2001). |
| 2002-2008  | Bangladesh    | O1 El tor ogawa, O1 | O139            | Fz ND                                           | Samal et al. (2001) |
| 2002      | Hubli, India  | O1 El Tor Ogawa, O139, Non-O1/non-O139 | O1 Ogawa: Amp (62.5), Co (81.3), NA/93.8/O139: Amp (100); Gent (54.5), Tet (54.5), NA (100); Non-O1/non-O139: Amp (82.4), Co (61.8), NA (94.1) | ND | Krishna et al. (2006) |
| 2001-2006  | East Delhi, India | O1 El Tor Ogawa/ Inaba | Co (96.6), Tet (97.3), Qu (4.2)                   | ND                                             | Das et al. (2008)    |
| Nov 2002-Apr 2004 | Mozambique | O1 El Tor Ogawa | Cm (57.9), Co (96.6), Tet (97.3), Qu (4.2) | ND | Mandomando et al. (2007) |
| 2003      | Thua thien, Vietnam | O1 Dhaka, Bangladesh, China | SXT, Tet, Ery, Sm Amp, Gent, Tet, Cm, SXT | ICE | Bani et al. (2007) |
| Sep 2004-Jun 2005 | Vietnam | O1 Dhaka | SXT, Tet, Ery, Sm Amp, Gent, Tet, Cm, SXT | SXT element pMRV150, pIP1202-like plasmid (IncA/C plasmid in MDR Y. pestis) | Furque et al. (2006) |
| 2004      | Chennai, India | O1 El Tor Ogawa (classical CTXW) | Co, NA, nitrofurantoin, Spec, Sm, SXT SXT, Sm, Cm | Class I integron SXT element | Goel et al. (2010) |
| 2004-2006  | Iran          | O1 El Tor Ogawa | SXT (100), Sm, Gent, Tet, Ery (44), SXT (99), Fz (100); Matlab: | SXT element | Adabi et al. (2009) |
| Oct 2004-Mar 2006 | Senega | O1 Cameron | Dhaka: Tet (55), Ery (44), SXT (99), Fz (100); Matlab: | ND | Manka et al. (2008) |
| 2004-2005  | Namibia       | O1 El Tor Ogawa and Inaba | Co, NA, nitrofurantoin, Spec, Sm, SXT | SXT element | Ngandjio et al. (2009) |
| 2005      | Iran          | O1 El Tor Inaba | SXT, Sm, Amp, co-amoxiclav, aztreonam, Co, Ery, metronidazole, NA, Neo, nitrofurantoin, oxacillin, PB, Spe, Sm, Tri, Vanc Inaba: NA (100), Amo (100), SXT (95.7), Fz (91.3), NAG: Ery (77.4) | ND | Keramat et al. (2008) |
| Aug 2006-Sep 2008 | North-west Ethiopia | O1 Inaba | SXT, Sm, Amp, co-amoxiclav, aztreonam, Co, Ery, metronidazole, NA, Neo, nitrofurantoin, oxacillin, PB, Spe, Sm, Tri, Vanc | SXT element | Opintan et al. (2008) |
| 2006      | Accra, Ghana  | O1              | SXT                                           | SXT element (88.9) Class 2 integron (81.5) Class 1 integron (7.4) | Nigdijio et al. (2009) |
| Dec 2006-Feb 2007 | Namibia | O1 El Tor Ogawa and Inaba | SXT, Sm, Amp, co-amoxiclav, aztreonam, Co, Ery, metronidazole, NA, Neo, nitrofurantoin, oxacillin, PB, Spe, Sm, Tri, Vanc Inaba: NA (100), Amo (100), SXT (95.7), Fz (91.3), NAG: Ery (77.4) | ND | Smith et al. (2008) |
| Aug-Sep 2007 | India        | O1 El Tor Ogawa and Inaba | SXT, Sm, Amp, co-amoxiclav, aztreonam, Co, Ery, metronidazole, NA, Neo, nitrofurantoin, oxacillin, PB, Spe, Sm, Tri, Vanc Inaba: NA (100), Amo (100), SXT (95.7), Fz (91.3), NAG: Ery (77.4) | ND | Jain et al. (2008) |
| 2008      | Iran          | O1 El Tor Inaba | Inaba: NA (100), Amo (100), SXT (95.7), Fz (91.3) | ND | Ranjbar et al. (2010) |
| Jun 2008-Jan 09 | Nepal | O1 El Tor Ogawa and Inaba | Fz (100), NA, Co Fz (99), Na, SXT | ND | Karki et al. (2010) |
| Jan 09    | Zimbabwe      | O1 El Tor Ogawa and Inaba | Fz, SXT                                         | ND                                             | Islam et al. (2009) |

However until these approaches will successfully implemented till we have to fall back on three key principles in managing this potentially deadly source: Clean water supplies, contaminant of cholera patient to stop transmission and use of oral rehydration therapy with antibiotics.
Acknowledgement

We wish to deeply thank Director MITS Gwalior for providing necessary facilities.

Funding Information

Actually this project is very low budget, hence all the financial and grants are arranged by department and institute. Therefore, no requirements of any grant agency and further financial support.

Author’s Contributions

Sabah Perveen: Concepts, definition of intellectual content, literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, guarantor.

Hotam Singh Chaudhary: Concepts, design, definition of intellectual content, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, manuscript review, guarantor.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Abera, B., B. Bezabih and A. Dessie, 2010. Antimicrobial susceptibility of V. cholerae in North West, Ethiopia. Ethiop Med. J., 48: 23-28.

Aberth, J., 2011. Plagues in World History. 1st Edn., Rowman and Littlefield Publishers, Lanham, ISBN-10: 1442207965, pp: 256.

Adabi, M., B. Bakhshi, H. Goudarzi, S.M. Zahraei and M.R. Pourshafie, 2009. Distribution of class I integron and sulfamethoxazole trimethoprim constin in Vibrio cholerae isolated from patients in Iran. Microbial Drug Resistance, 15: 179-184.

Allen, J.G., F.R. Atherton, M.J. Hall, C.H. Hassall and S.W. Holmes et al., 1979. Phosphonopetides as antibacterial agents: Alaphospin and related phosphonopeptides. Antimicrobial Agents Chemotherapy, 15: 684-695.

Atherton, F.R., M.J. Hall, C.H. Hassall, R.W. Lambert and W.J. Lloyd et al., 1979. Phosphonopeptides as antibacterial agents: Mechanism of action of alaphospin. Antimicrobial Agents Chemotherapy, 15: 696-670.
Daccord, A., D. Cecarelli and V. Burrus, 2010. Integrating conjugative elements of the SXT/R391 family trigger the excision and drive the mobilization of a new class of Vibrio genomic islands. Mol. Microbiol., 78: 576-588. DOI: 10.1111/j.1365-2958.2010.07364.x

Das, S., R. Saha and I.R. Kaur, 2008. Trend of antibiotic resistance of Vibrio cholerae strains from East Delhi. Indian J. Med. Res., 127: 478-482. PMID: 18653912

Dziejman, M., E. Balon, D. Boyd, C.M. Fraser and J.F. Das, S., R. Saha and I.R. Kaur, 2008. Trend of antibiotic resistance of Vibrio cholerae strains from East Delhi. Indian J. Med. Res., 127: 478-482. PMID: 18653912

Ehara, M., B.M. Nguyen, D.T. Nguyen, C. Toma and D. Ceccarelli and V. Burrus, 2010. Fasano, A., B. Baudry, D.W. Pumplin, S.S. Wasserman. Molecular Biology of Antimicrobial Drug Action. 6th Edn., Springer, New York, ISBN-10: 0387225544, pp: 182.

Finkelstein, R.A. and J.J. LoSpalluto, 1969. Pathogenesis of experimental cholera preparation and isolation of choleraugen and choleraugenoid. J. Experimental Med., 130: 185-202. DOI: 10.1084/jem.130.1.185

Gardner, A.D. and K.V. Venkatramakn, 1935. The antigens of the cholera group of vibrios. J. Hyg., 35: 262-282.

Gellert, M., K. Mizuuchi, M.H. O’Dea, T. Itoh and J.I. Tomizawa, 1977. Nalidixic acid resistance: A second genetic character involved in DNA gyrase activity. Proc. National Acad. Sci., USA, 74: 4772-4776. PMID: 337300

Glass, R.I., I. Huq, A.R.M.A. Alim and M. Yunus, 1980. Emergence of multiply antibiotic-resistant Vibrio cholerae in Bangladesh. J. Infect. Dis., 142: 939-942. DOI: 10.1093/infdis/142.6.939

Goel, A.K., M. Jain, P. Kumar and S.C. Jiang, 2010. Molecular characterization of Vibrio cholerae outbreak strains with altered El Tor biotype from southern India. World J. Microbiol. Biotechnol., 26: 281-287. DOI: 10.1007/s11274-009-0171-7

Hannan, A., T. Humayun, B.M. Hussain, M. Yasir and S. Sikandar, 2010. In vitro antibacterial activity of onion (Allium cepa) against clinical isolates of Vibrio cholerae. J. Ayub. Med. Abbottabad, 22: 160-163. PMID: 21702293

Hör, M., H. Rimpler and M. Heinrich, 1995. Inhibition of intestinal chloride secretion by proanthocyanidins from Guazuma ulmifolia. Planta Med., 61: 208-212. DOI: 10.1055/s-2006-958057

Huda, M.N., J. Chen, Y. Morita, T. Kuroda and T. Goel, A.K., M. Jain, P. Kumar and S.C. Jiang, 2010. Molecular characterization of Vibrio cholerae outbreak strains with altered El Tor biotype from southern India. World J. Microbiol. Biotechnol., 26: 281-287. DOI: 10.1007/s11274-009-0171-7

Hung, D.T., E.A. Shakhnovich, E. Pierson and J.J. Tomizawa, 1977. Nalidixic acid resistance: A second genetic character involved in DNA gyrase activity. Proc. National Acad. Sci., USA, 74: 4772-4776. PMID: 337300

Hudson, T. and M. Waldor, 2000. Mobilization of plasmids and chromosomal DNA mediated by the SXT element, a constin found in Vibrio cholerae O139. J. Bacteriol., 182: 2043-2047. DOI: 10.1128/JB.182.7.2043-2047.2000

Islam, M.S., S.M. Midzi, L. Charimari, A. Cravioto and H.P. Endtz, 2009. Susceptibility to fluoroquinolones of Vibrio cholerae O1 isolated from diarrheal patients in Zimbabwe. JAMA, 302: 2321-2322. DOI: 10.1001/jama.2009.1750

Hochlut, B., J. Marrero and M.K. Waldor, 2000. Mobilization of plasmids and chromosomal DNA mediated by the SXT element, a constin found in Vibrio cholerae O139. J. Bacteriol., 182: 2043-2047. DOI: 10.1128/JB.182.7.2043-2047.2000

Hochlut, B., J. Marrero and M.K. Waldor, 2000. Mobilization of plasmids and chromosomal DNA mediated by the SXT element, a constin found in Vibrio cholerae O139. J. Bacteriol., 182: 2043-2047. DOI: 10.1128/JB.182.7.2043-2047.2000

Hochlut, B., J. Marrero and M.K. Waldor, 2000. Mobilization of plasmids and chromosomal DNA mediated by the SXT element, a constin found in Vibrio cholerae O139. J. Bacteriol., 182: 2043-2047. DOI: 10.1128/JB.182.7.2043-2047.2000

Hochlut, B., J. Marrero and M.K. Waldor, 2000. Mobilization of plasmids and chromosomal DNA mediated by the SXT element, a constin found in Vibrio cholerae O139. J. Bacteriol., 182: 2043-2047. DOI: 10.1128/JB.182.7.2043-2047.2000
Kumar, P., M. Jain, K.A. Goe, V.D. Kamboj and O. Kumar, 2012. Tetracycline resistant V. cholerae O1 biotype El Tor serotype Ogawa with classical ctxB from a recent cholera outbreak in Orissa, Eastern India. J. Infect. Public Health, 5: 217-219. DOI: 10.1016/j.jiph.2011.09.007

Kunin, C.M., 1993. Resistance to antimicrobial drugs a worldwide calamity. Ann. Int. Med., 118: 557-561. DOI: 10.7326/0003-4819-118-7-19930410-00011

Mandomdo, I., M. Espasa, X. Valles, J. Sacarlal and B. Sigaquue et al., 2007. Antimicrobial resistance of Vibrio cholerae O1 serotype Ogawa isolated in manihca district hospital, southern Mozambique. J. Antimicrobial Chemotherapy, 60: 662-664. DOI: 10.1093/acidbm/ekm257

Manga, N.M., C.T. Ndour, S.A. Diop, N.M. Dia and R. Ka-Sall et al., 2008. Cholera in Senegal from 2004 to 2006: Lessons learned from successive outbreaks. Med. Trop. (Mars.), 68: 589-592. PMID: 19639824

Martinez, J.L., 2008. Antibiotics and antibiotic resistance genes in natural environments. Science, 321: 365-367. DOI: 10.1126/science.1159483

Mazel, D., 2006. Integrons: Agents of bacterial evolution. Nature Rev. Microbiol., 4: 608-620. DOI: 10.1038/nm1462

Mekalanos, J.J., E.J. Rubin and M.K. Waldor, 1997. Cholera: Molecular basis for emergence and pathogenesis. FEMS Immun. Med. Microbiol., 18: 241-248. DOI: 10.1111/j.1574-695X.1997.tb01052.x

Ngandjio, A., M. Tejiokem, M. Wouafo, I. Ndome and M. Yonga et al., 2009. Antimicrobial resistance and molecular characterization of Vibrio cholerae O1 during the 2004 and 2005 outbreak of cholera in Cameroon. Foodborne Pathogens Dis., 6: 49-56. DOI: 10.1089/fpd.2008.0127

Oi, H., D. Matsuura, M. Miyake, M. Ueno and I. Takai et al., 2002. Identification in traditional herbal medications and confirmation by synthesis of factors that inhibit cholera toxin-induced fluid accumulation. Proc. Nat. Acad. Sci. USA, 99: 3042-3046. DOI: 10.1073/pnas.052709499

Opintan, J.A., M.J. Newman, O.A. Nsiah-Poodoh and I.N. Okeke, 2008. Vibrio cholerae O1 from Accra, Ghana carrying a class 2 integron and the SXT element. J. Antimicrobial Chemotherapy, 62: 929-933. DOI: 10.1093/jac/dkn334

Paulsen, I.T., M.H. Brown and R.A. Skurray, 1996. Proton dependent multidrug efflux systems. Microbiol. Mol. Biol. Rev., 60: 575-608.

Pearson, G.D., A. Woods, S.L. Chiang and J.J. Mekalanos, 1993. CTX genetic element encodes a site-specific recombination system and an intestinal colonization factor. Proc. National Acad. Sci., 90: 3750-3754. DOI: 10.1073/pnas.90.8.3750
Politi, M., J. Alvaro-Blanco, P. Groves, A. Prieto and J.A., Leal et al., 2006. Screening of garlic water extract for binding activity with cholera toxin B pentamer by NMR spectroscopy—an old remedy giving a new surprise. Eur. J. Organic Chem., 2006: 2067-2073. DOI: 10.1002/ejoc.200500875

Provenzano, D., P. Kovac and W.F. Wade, 2006. The ABCs (Antibody, B cells and Carbohydrate epitopes) of cholera immunity: Considerations for an improved vaccine. Microbiol. Immunol., 50: 899-927. DOI: 10.1111/j.1348-0421.2006.tb03866.x

Pan, J.C., R. Ye, H.Q. Wang, H.Q. Xiang and W. Zhang et al., 2008. Vibrio cholerae O139 multiple-drug resistance mediated by Yersinia pestis pIP1202-like conjugative plasmids. Antimicrob Agents Chemother, 52: 3829-3836. DOI: 10.1128/AAC.00375-08

Rahim, N., D.J. Gomes, H. Watanabe, S.R. Rahman and A. Golmohamadi, 2008. Antibacterial activity of Psidium guajava leaf and bark against multidrug-resistant Vibrio cholerae: Implication for cholera control. Japanese J. Infect. Dis., 63: 271-274. PMID: 20657067

Rakoto, A.AO, J.A. Dromigny, P. Pfister and P. Maucere, 2001. Vibrio cholerae in Madagascar: Study of a multiresistant strain. Arch. Inst. Pasteur Madagascar, 67: 6-13. PMID: 12471739

Ranjbar, M., E. Rahmani, A. Nooriamiri, H. Gholami et al., 2010. High prevalence of multidrug-resistant strains of Psidium guajava leaf and bark against multidrug-resistant Vibrio cholerae: Implication for cholera control. Japanese J. Infect. Dis., 63: 271-274. PMID: 20657067

Rhine, J.A. and R.K. Taylor, 1994. TcpA pilin sequences and colonization requirements for O1 and O139 Vibrio cholerae. Molecular Microbiol., 13: 1013-1020. DOI: 10.1111/j.1365-2958.1994.tb00942.x

Roychowdhury, A., A. Pan, D. Dutta, A.K. Mukhopadhyay and T. Ramamurthy et al., 2008. Emergence of tetracycline-resistant Vibrio cholerae O1 serotype Inaba, in Kolkata, India. Jpn. J. Infect. Dis., 61: 128-129. PMID: 18362401

Sack, D.A., R.B. Sack, G.B. Nair and A.K. Siddique, 2004. Cholera. Lancet, 363: 223-233. DOI: 10.1016/S0140-6736(03)15328-7

Saito, T., M. Miyake, M. Toba, H. Okamatsu and S. Shimizu et al., 2002. Inhibition by apple polyphenols of ADP-ribosyltransferase activity of cholera toxin and toxin-induced fluid accumulation in mice. Microbiol. Immunol., 46: 249-255. DOI: 10.1111/j.1348-0421.2002.tb02693.x

Samal, B., S.K. Ghosh, S.K. Mohanty and K. Patnaik, 2001. Epidemic of Vibrio cholerae serogroup O139 in Berhampur, Orissa. Indian J. Med. Res., 114: 10-11. PMID: 11762200

Sedas, V.T., 2007. Influence of environmental factors on the presence of Vibrio cholerae in the marine environment: A climate link. J. Infect. Develop. Countries, 1: 224-241. PMID: 19734600

Shimada, T., E. Arakawa, K. Itoh, T. Okitsu and A. Matsushima et al., 1994. Extended serotyping scheme for Vibrio cholerae. Current Microbiol., 28: 175-178. DOI: 10.1007/BF01371061

Shittu, O.B., O.O. Olabode, A.M. Omem, S.A. Oluwalana and S. Adeniran et al., 2014. Evaluation of Spondias mombin, Senna occidentalis and Musa sapientum against Vibrio cholerae O1 in experimental mice. Int. J. Curr. Microbiol. Applied Sci., 3: 975-995.

Singh, P., S. Mishra and H. Sharma, 2013. To study the therapeutic role of Indian spices in the treatment of gastrointestinal disease caused by Vibrio species. Int. J. Innovative Res. Sci. Eng. Technol., 2: 2371-2375.

Smith, A.M., K.H. Keddy and L. De Wee, 2008. Characterization of cholera outbreak isolates from Namibia. Epidemiol. Infect., 136: 1207-1209. DOI: 10.1017/S0950268807009685

Sugino, A., C.L. Peebles, K.N. Kreuzer and N.R. Cozzarelli, 1977. Mechanism of action of nalidixic acid: Purification of Escherichia coli nalA gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. National Acad. Sci., USA, 74: 4767-4771. PMID: 209930

Tjianiadi, P., M. Lesmana, D. Subekti, N. Machpud and S. Komalarini et al., 2003. Antibimicrobial resistance of bacterial pathogens associated with diarrheal patients in Indonesia. Hygiene, 68: 666-670. PMID: 12887025

Trucksis, M., J.E. Galen, J. Michalski, A. Fasano and J.B. Kaper, 1993. Accessory Cholera Enterotoxin (ACE), the third toxin of a Vibrio cholerae virulence cassette. Proc. National Acad. Sci., 90: 5267-5271. PMID: 8394746

Waldor, M.K., H. Tschape and J.J. Mekalanos, 1996. A novel nicking-closing enzyme. Proc. National Acad. Sci., USA, 74: 4767-4771. PMID: 209930

Yamai, S., T. Okitsu, T. Shimada and Y. Katsube, 1997. Study of a multiresistant strain. Arch. Inst. Pasteur Madagascar, 67: 6-13. PMID: 12471739

S. Komalarini et al., 2008. Vibrio cholerae O139 multiple-drug resistance efflux operon in Vibrio cholerae O139. J. Bacteriol., 178: 4157-4165.

Trucksis, M., J.E. Galen, J. Michalski, A. Fasano and J.B. Kaper, 1993. Accessory Cholera Enterotoxin (ACE), the third toxin of a Vibrio cholerae virulence cassette. Proc. National Acad. Sci., 90: 5267-5271. PMID: 8394746

Waldor, M.K., H. Tschape and J.J. Mekalanos, 1996. A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim and streptomycin in Vibrio cholerae O139. J. Bacteriol., 178: 4157-4165. DOI: http://jb.asm.org/content/178/4/4157.short

Woolley, R.C., G. Vediyappan, M. Anderson, M. Lackey and B. Ramasubramanian et al., 2005. Characterization of the Vibrio cholerae vceCAB multiple-drug resistance efflux operon in Escherichia coli. J. Bacteriol., 187: 5500-5503. DOI: 10.1128/JB.187.15.5500-5503.2005

Yamai, S., T. Okitsu, T. Shimada and Y. Katsube, 1997. Distribution of serogroups of Vibrio cholerae non-O1 non-O139 with specific reference to their ability to produce cholera toxin and addition of novel serogroups. J. Japanese Assoc. Infect. Dis., 71: 1037-1045. PMID: 9394556