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Review

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

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Corresponding Author:
Hotam Singh Chaudhary
Department of Biotechnology,
Madhav Institute of
Technology and Science
Gwalior, RGPV, India
Email: hotamsingh@gmail.com

Abstract: *Vibrio cholerae* is a causative agent of *cholerae*, 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. But 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; Karaolis *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 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 atleast 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 gens (ctxAB) are located on CTXφ phage and they both are under control of transcriptional activator ToxT.

Treatment

Earlier lytic bacteriophages were used for the treating the cholera when the molecular biology and antibiotics not discovered in India in late 1920s and early 1930s Summers, 1993. Oral Rehydration Salt (ORS) is primarily recommended for the treatment of cholera. In severe cases antibiotics are used to treat *cholerae* either in combination with ORS. Many antibiotics used against *V. cholerae* such as doxycycline, cotrimoxazole, erythromycin, tetracycline, chloramphenicol, furazolidone, etc. Doxycycline is used as first line treatment in adults. Whereas azithromycin is recommended as first-line treatment for children and pregnant women. Tetracycline, chloramphenicol these antibiotics inhibit the protein synthesis in bacteria (Franklin and Snow, 2005). Ciprofloxacin (1g orally in 1dose) and doxycycline (300 mg in 1dose) are the antibiotics choose for adult except pregnant women.

The reason behind the increasing drug resistant is undoubtedly the immense amount of antibacterial drug used in the treatment of humans and animals worldwide. It's now became necessary to minimize the unnecessary use of antibacterial drugs, in order to control the resistance. However now the need is to find a renewed antibiotics and new drug targets. It became resistant through various mechanism like exporting drugs through efflux pump, chromosomal mutation or developing genetic resistant via the exchange of conjugative plasmids, conjugative transposons, integrons or self transmissible chromosomally integrating SXT elements (Kitaoka *et al.*, 2011).

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 cholera epidemic in the United Republic of Tanzania, the rate of mutation in *V. cholerae* genes is higher than the *E. coli* genes. This facilitates the resistance to antibiotics such as alafosfalin (Atherton *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 *V. cholerae*. There are various reports documented the multi-drug resistance in *V. cholerae* including the antibiotics tetracycline, erythromycin, chloramphenicol, quinolones, streptomycin and cotrimoxazole (Abera *et al*., 2010; Das *et al*., 2008; Islam *et al*., 2009; Karki *et al*., 2010; Ngandjio *et al*., 2009; Ranjbar *et al*., 2010; Roychowdhury *et al*., 2008; Kumar *et al*., 2014; 2012).

SXT Elements and Integrons

The SXT elements was first described in *V. cholerae* serogroup O139 based on its ability to harbor genes which provide the host bacterium with resistant to sulfamethoxazle, trimethoprim and streptomycine (Waldor *et al*., 1996). Beaber *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 conjucation 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 *et al*., 2010; Hochhut *et al*., 2000), providing an alternative mechanism for antibiotic resistant gene transfer. All *V. cholerae* isolates facilitates the large chromosomal integrons that provide them the capacity to rapidly transfer gene cassettes containing antibiotic resistant genes (Mazel, 2006).

Conjugative Plasmids

Many *V. cholerae* strains are identified which developed resistant against tetracycline antibiotics, an oral drug often given to patient during rehydration therapy (Greenough *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 *et al.*, 2011). This resistance was transferable to 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 *E.coli* (Glass *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 *V. cholerae* became resistant to antibiotics. Now the main target of present day is to find an alternative for these diseases.

Herbal Plants- good antimicrobial Activity: There are many herbal plants presents 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 *V. cholerae* from various medicinal plants. The active compounds present in natural compound can be used to treat *V. cholerae* by various pharmacologic mechanisms. Some compound shows direct antimicrobial activity against *V. cholerae* and some inhibit the binding of CT to GM receptors at epithelial cell surface. On this mechanism many herbal compounds identified against *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 S. aureus, *S. typhosa* (Chaurasia and Jain, 1978) *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 *et al*. (2010) showed that *Allium cepa* has antimicrobial property and it inhibit the growth of *V. cholerae*. They found that the antimicrobial activity of purple type of allium cepa extract was better as compared to yellow *Allium cepa* axtract
- Indian Species: Praveen Singh *et al*. (2013), has found that many Indian species showed antimicrobial property against *V. cholerae. The* extract of extracts of Black cardamom *(Amomum subulatum),* Mustard seed *(Brassica nigra)* , Red Chilli *(Capsicum annuum),* Bay leaf *(Cinnamomum tamala),* Cinnamon *(Cinnamomum verum),* Coriander seed *(Coriandrum sativum),* Cumin seed *(Cuminum cyminum),* Green cardamom *(Elettaria cardamomum),* Liquorice (*Glycyrrhiza glabra*), carom seed/Thyme (*Trachyspermum ammi)* , Anise *(Pimpinella anisum)* , Black pepper *(Piper nigrum),* Fenugreek *(Trigonella foenum-graecum),* Turmeric *(Curcuma longa),* Dry ginger *(Zingiber officinale)* showed a significant level of antimicrobial activity against *V. cholerae*
- *Cocculus hirsutus Linn*: Kalirajan *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 aquous 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 polysaccharide a bioactive compound present in garlic extract as a mojor 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 nautral 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 *Guazumaulimfolia*, 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 compound capsaisin present in red chilli act as antimicrobial agent against many pathogenic bacteria *V. cholerae*, bacillus sps. 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 sapeintum* 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 (Martinez, 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 (3)	ND	Jabeen *et al* (2008)
1995-2001	Indonesia	O1/non-O1	Amp, SXT, Cm, Tet	ND	Tjaniadi *et al* (2003)
1995,2000, 2002	Vietnam	O1	1995: Sm; 2000: SXT, Sm; 2000:--	1995: Class I integrons aadA1, 2000 SXT element, str AB2002:---	Ehara *et al*. (2004)
Jan 1999-Dec 2007	India	O1 E1 tor ogawa	Fz, Cpr, Amo, Co	ND	Chander *et al.* (2009).
2000-2004	Hubli, India	O1, O139, non-O1, non-O139	Fq (since 2002)	ND	Chandrasekhar *et al*. (2008)
2000	Madagascar	ND	Co, Sm, Cm, Amp, Tet	26Kb self-transmissible plasmid	Rakoto *et al*. (2001)\
May-June 2000	India	O1 E1 tor ogawa,	O139	Fz ND	Samal *et al*. (2001)
2002-2008	Bangladesh	O1	Cpr	qnrVC3 encoded on SXT element protect topoisomerase from quinolone	Kim *et al*. (2010)
2002	Hubli, India	O1 E1 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 E1 Tor Ogawa/ Inaba	NA, Co	ND	Das *et al*. (2008)
Nov2002-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	Amo, Ery	ICE	Bani *et al*. (2007)
Sep 2004-Jun 2005	Dhaka, Bangladesh	O1	SXT, Tet, Ery, Sm	SXT element	Faruque *et al*. (2006)
2004	Hangzhou, East China	O139	Amp, Sm, Gent, Tet, Cm, SXT	pMRV150; pIP1202-like plasmid (IncA/C plasmid in MDR Y. pestis)	Pan *et al*. (2008)
2004	Chennai, India	O1 El Tor Ogawa (classical CTXW)	Co, NA, nitrofurantoin, Spec, Sm, SXT	Class I integron SXT element	Goel *et al*. (2010)
2004-2006	Iran	ND	SXT, Sm, Cm	SXT element	Adabi *et al*. (2009)
Oct 2004-Mar 2006	Senega	O1 El Tor	Co (90.3)	ND	Manga *et al*. (2008)
2004-2005	Cameroon	O1	SXT (100), Amp	ND	Ngandjio *et al*. (2009)
Oct 2004-Dec 2005	Dhaka and Matlab, Bangladesh	O1 Ogawa and Inaba	Dhaka: Tet (55), Ery (44), SXT (99), Fz (100): Matlab: Tet (54), Ery (48), Ery (97), Fz (100)	ND	Faruque *et al*. (2007)
2005	Iran	O1 El Tor Inaba	Nf (97), Cpr (92), Kan (88),amikacin (85), Tet (77), Dox (67), Fz (100), SXT (98), Ery (62)	ND	Keramat *et al*. (2008)
Aug 2006-Sep 2008	North-west Ethiopia	O1 Inaba	Co (100), Cm (94), Amp (89), Ery (15), Tet (6.2), Cpr (1.2)	ND	Abera *et al*. (2010)
2006	Accra, Ghana	O1	SXT	SXT element (88.9) Class 2 integron (81.5) Class 1 integron (7.4)	Opintan *et al*. (2008)
Dec 2006-Feb 2007	Namibia	Namibia	SXT, Sm	ND	Smith *et al*. (2008)
Aug-Sep 2007	India	O1 El Tor	Amp, co-amoxiclav, aztreonam, Co, Ery, metronidazole, NA, Neo, nitrofurantoin, oxacillin, PB, Spe, Sm, Tri, Vanc	Class 1 integron SXT element	Jain *et al*. (2008)
2008	Iran	O1 El Tor Inaba Non-agglutinating (NAG) strains	Inaba: NA (100), Amo (100), SXT (95.7), Fz (91.3) NAG: Ery (77.4)		Ranjbar *et al*. (2010)
Jun 2008-Jan-09	Nepal	O1 El Tor Ogawa	Fz (100), NA, Co	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.

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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.

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