Antibiotic resistance mechanisms of *Vibrio cholerae*

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As the causative agent of cholera, the bacterium *Vibrio cholerae* represents an enormous public health burden, especially in developing countries around the world. Cholera is a self-limiting illness; however, antibiotics are commonly administered as part of the treatment regimen. Here we review the initial identification and subsequent evolution of antibiotic-resistant strains of *V. cholerae*. Antibiotic resistance mechanisms, including efflux pumps, spontaneous chromosomal mutation, conjugative plasmids, SXT elements and integrons, are also discussed. Numerous multidrug-resistant strains of *V. cholerae* have been isolated from both clinical and environmental settings, indicating that antibiotic use has to be restricted and alternative methods for treating cholera have to be implemented.

### Introduction

The acute diarrhoeal disease cholera is responsible for approximately 120 000 deaths every year and has a major impact on the health of young children between the ages of 1 and 5 years (WHO, 1995). Cholera is contracted by ingestion of food or water contaminated with the Gram-negative bacterium *Vibrio cholerae*. The bacteria pass through the human gastric acid barrier into the small intestine where they colonize, multiply and begin to secrete cholera toxin. Because this organism is sensitive to the low pH found in the human stomach, a high infectious dose of ~10⁸ bacteria is required for the onset of severe cholera; however, the infectious dose can drop to ~10⁴ bacteria in stool, are shed daily containing 10⁹ bacteria is required for the onset of severe cholera; however, the infectious dose can drop to ~10⁴ bacteria in individuals who produce less stomach acid, including young children, the elderly and those who take antacids (Cash et al., 1974). About 1–5 days after ingestion, cholera patients experience sudden watery diarrhoea and vomiting. Up to 20 litres of watery diarrhoea, referred to as rice-water stool, are shed daily containing 10⁹ *V. cholerae* per millilitre of stool (Dizon et al., 1967). Water loss caused by cholera may reach one litre per hour in adults, leading to severe dehydration, shock and eventual death (Sack et al., 2004). If left untreated, the cholera fatality rate reaches 50% within a few hours to days after onset of the disease (Maier & Pepper, 2009).

*V. cholerae* utilizes two virulence factors, the toxin coregulated pilus (TCP) and cholera toxin. TCP, encoded in the *Vibrio* pathogenicity island I (VPI I), is a type IV pilus that allows the organism to aggregate (Kirn et al., 2000) – a mechanism that protects individual cells from shearing forces in the small intestine. TCP is also essential for *V. cholerae* colonization of the small intestine of infant mice (Taylor et al., 1987) and humans (Herrington et al., 1988). Once the small intestine is successfully colonized, *V. cholerae* cells secrete cholera toxin. The toxin activates the cystic fibrosis transmembrane conductance regulator (CFTR) in the epithelial cells that line the small intestine, leading to massive fluid efflux into the lumen of the small intestine.

Over 200 *V. cholerae* O-antigen serogroups have been identified (Shimada et al., 1994). With the exception of O139 (Siddique et al., 1996), strains of the O1 serogroup have been responsible for all of the major cholera pandemics recorded so far. O1 strains are further classified into ‘classical’ and ‘El Tor’ biotypes based on phenotypic characteristics (listed in Table 1) and defined genotypic differences (Safa et al., 2010).

The first six recorded cholera pandemics (1899–1923) have been attributed to classical strains (Safa et al., 2010), while El Tor strains are responsible for the current seventh pandemic, which started in 1961 in Indonesia (Colwell, 1996). In the early 1990s, around the same time as the O139 strain emerged, new variants of *V. cholerae* O1

### Table 1. Phenotypic differences between *V. cholerae* classical and El Tor O1 strains

<table>
<thead>
<tr>
<th>Test</th>
<th>Classical</th>
<th>El Tor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolysis of sheep erythrocytes</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Agglutination of chicken erythrocytes</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Voges–Proskauer reaction</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Polymyxin B resistance</td>
<td>s</td>
<td>r</td>
</tr>
<tr>
<td>Phage IV</td>
<td>s</td>
<td>r</td>
</tr>
<tr>
<td>Phage 5</td>
<td>r</td>
<td>s</td>
</tr>
</tbody>
</table>

A number of tests can be used to distinguish biotypes, including haemolysis of sheep erythrocytes, agglutination of chicken erythrocytes, the Voges–Proskauer reaction (detects the presence of acetoin in bacterial cultures) and sensitivity to polymyxin B and to specific bacteriophages. +, Positive; –, negative; s, sensitive; r, resistant.
appeared carrying both classical and El Tor biotype traits (hybrid strains) (reviewed by Safa et al., 2010).

All *V. cholerae* serogroups can be found in aquatic environments such as estuaries and brackish waters (Kaper et al., 1979) as either free-living cells or associated with other aquatic organisms such as copepods (Huq et al., 1983). Upon nutrition deprivation (likely to be encountered in aquatic environments), *V. cholerae* switches to a viable-but-nonculturable form which cannot be grown under standard culturing conditions. Quiescent *V. cholerae* reverts back to the infectious, transmissible state when the organism encounters favourable conditions (Binsztein et al., 2004). Quiescence is believed to contribute to the persistence of *V. cholerae* in aquatic environments between cholera epidemics (Reidl & Klose, 2002). Genetic exchange between *V. cholerae* and other bacteria or viruses is responsible for the emergence of toxigenic strains (Mekalanos et al., 1997; Faruque et al., 2005).

**Why use antibiotics?**

*V. cholerae* does not typically cause systemic infection. Antibiotics are not required to resolve cholera symptoms and cannot be used as a sole treatment for the disease; however, there are advantages to combining oral rehydration therapy with antibiotic treatment (antibiotics discussed here are listed in Table 2). Antibiotics are administered to lessen the duration of illness by approximately 50% and to reduce shedding of *V. cholerae* in the stool (Greenough et al., 1964; Lindenbaum et al., 1967; Pierce et al., 1968). Antibiotics reduce the severity of symptoms by decreasing the volume of diarrhoea, and thus the amount of fluids required to maintain hydration (Greenough et al., 1964; Lindenbaum et al., 1967; Pierce et al., 1968). This is especially important in developing countries where access to safe drinking water is limited and oral rehydration solutions are in short supply.

For the treatment of cholera, an oral or intravenously administered solution containing glucose, sodium chloride, potassium chloride and trisodium citrate can save a patient from dehydration (WHO, 2002). The antibiotics tetracycline and quinolones have been widely used (Mhalu et al., 1979; Towner et al., 1980) to reduce the symptoms of cholera, but the emergence of *V. cholerae* strains resistant to antibiotics has restricted their use to patients with severe dehydration (Garg et al., 2001). In severe cases, a single dose of doxycycline (a member of the tetracycline antibiotics group) co-administered with fluid replacement therapy is usually sufficient to stabilize the patient. Alternatively, a multidose treatment of tetracycline can be administered; in the case of young children, liquid erythromycin is preferred (WHO, 2004). In a randomized clinical trial, erythromycin yielded the best clinical recovery rates in children (Roy et al., 1998).

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Class</th>
<th>Compounds</th>
<th>Bacterial target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell growth inhibitors</td>
<td>β-Lactam antibiotics</td>
<td>Penicillin, ampicillin</td>
<td>Penicillin-binding protein</td>
</tr>
<tr>
<td>Glycopeptide</td>
<td>Vancomycin</td>
<td>Nascent peptidoglycan</td>
<td></td>
</tr>
<tr>
<td>Phosphonopeptides</td>
<td>Alafosfalin</td>
<td>Alanine racemase</td>
<td></td>
</tr>
<tr>
<td>Macrolides</td>
<td>Erythromycin</td>
<td>50S subunit of the ribosome</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>–</td>
<td>50S subunit of the ribosome</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Streptomycin, kanamycin, gentamicin</td>
<td>30S subunit of the ribosome</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>–</td>
<td>30S subunit of the ribosome</td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline, doxycycline</td>
<td>30S subunit of the ribosome</td>
<td></td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td>Linezolid</td>
<td>23S subunit of the ribosome</td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>–</td>
<td>β-Subunit of RNA polymerase</td>
<td></td>
</tr>
<tr>
<td>Folic acid metabolism inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>Sulfamethoxazole</td>
<td>Dihydropteroate synthase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
<td>Dihydrofolic acid reductase</td>
<td></td>
</tr>
<tr>
<td>DNA replication inhibitors</td>
<td>(Fluoro)quinolones</td>
<td>Ciprofloxacin, nalidixic acid, norfloxacin</td>
<td>Topoisomerase II (DNA gyrase); topoisomerase IV</td>
</tr>
<tr>
<td>Inducers of cell lysis/cytotoxicity</td>
<td>Polymyxins</td>
<td>Polymyxin B</td>
<td>Cell membrane</td>
</tr>
<tr>
<td>Anthracyclines</td>
<td>Doxorubicin</td>
<td>Inhibition of topoisomerase II; binding to DNA and membranes; generation of toxic radicals</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Antibiotics (discussed in this review) and their mode of action**

Information collected from Katzung et al. (2009), except for alafosfalin (Atherton et al., 1979).
Though there are obvious benefits to individuals who are treated with antibiotics, the World Health Organization does not recommend their general use because antibiotics contribute to increasing antimicrobial resistance, making cholera and other bacterial infections more difficult to treat (Glass et al., 1980; Hedges & Jacob, 1975; Sack et al., 2001; Threlfall & Rowe, 1982). Use of antibiotics to treat cholera should be strictly relegated to patients suffering from severe dehydration.

**Mechanisms of antibiotic resistance**

*V. cholerae* becomes drug resistant by exporting drugs through efflux pumps, chromosomal mutations or developing genetic resistance via the exchange of conjugative plasmids, conjugative transposons, integrons or self-transmissible chromosomally integrating SXT elements.

Infections caused by antibiotic-resistant *V. cholerae* from 2000 to 2010 are listed in Table 3.

**Bacterial efflux pumps**

*V. cholerae* uses multidrug efflux pumps to export a broad range of antibiotics, detergents and dyes that are chemically and structurally unrelated (Paulsen et al., 1996). The two major groups of *V. cholerae* efflux pumps are distinguished by their energy sources: ATP hydrolysis, or the proton-motive force (PMF) of transmembrane H\(^+\) or Na\(^+\) gradients (Putman et al., 2000). PMF pump families include MATE (multidrug and toxic compound extrusion), MFS (major facilitator superfamily), RND (resistance–nodulation–cell division) and SMR (small multidrug resistance) (Paulsen et al., 1996). One of the few bacterial ATP-driven pumps is VcaM, a *V. cholerae* ATPase.

**Fig. 1.** Important historical events related to *Vibrio cholerae* and the pandemics. Key breakthroughs in the field are listed on the left side of the bar. Pandemics are indicated as coloured columns with the responsible serogroups indicated within. Selected outbreaks are listed on the right side of the figure. Photographs are public domain according to the ‘Images from the History of Medicine (IHM)’ and Wikimedia Commons.
Table 3. Major drug-resistant *V. cholerae* strains reported in the last decade

Numbers in parentheses indicate the percentage of *V. cholerae* isolates with antibiotic resistance. ND, Not determined; Amo, amoxicillin; Amp, ampicillin; Cm, chloramphenicol; Co, cotrimoxazole; Cpr, ciprofloxacin; Dox, doxycycline; Ery, erythromycin; FQ, fluoroquinolone; Fz, furazolidone; Gent, gentamicin; Kan, kanamycin; NA, nalidixic acid; Neo, neomycin; Nf, norfloxacin; PB, polymyxin B; Qu, quinolone; Sm, streptomycin; Spec, spectinomycin; SXT, sulfamethoxazole–trimethoprim; Su, sulphonamides; Tet, tetracycline; Tri, trimethoprim; Vanc, vancomycin.

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Strain</th>
<th>Antibiotic resistance</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993–2005</td>
<td>Pakistan</td>
<td>O1 Inaba/Ogawa</td>
<td>Co (100), Cm (3)</td>
<td>ND</td>
<td>Jabeen <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>1995–2001</td>
<td>Indonesia</td>
<td>O1/non-O1</td>
<td>Amp, SXT, Cm, Tet</td>
<td>ND</td>
<td>Tjaniadi <em>et al.</em> (2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2002: Sm</td>
<td>2002: −</td>
<td></td>
</tr>
<tr>
<td>Jan 1999–Dec</td>
<td>India</td>
<td>O1 El Tor Ogawa</td>
<td>Fz, Cpr, Amo, Co</td>
<td>ND</td>
<td>Chander <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Madagascar</td>
<td>ND</td>
<td>Co, Sm, Cm, Amp, Tet</td>
<td>26 kb self-transmissible plasmid</td>
<td>Rakoto Alson <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>May–Jun 2000</td>
<td>India</td>
<td>O1 El Tor Ogawa O139</td>
<td>Fz</td>
<td>ND</td>
<td>Samal <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>2002–2008</td>
<td>Bangladesh</td>
<td>O1</td>
<td>Cpr</td>
<td>* gyrVC3* encoded on SXT element protects topoisomerase from quinolone</td>
<td>Kim <em>et al.</em> (2010)</td>
</tr>
<tr>
<td>2002</td>
<td>Hubli, India</td>
<td>O1 El Tor Ogawa O139 Non-O1/non-O139</td>
<td>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)</td>
<td>ND</td>
<td>Krishna <em>et al.</em> (2006)</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov 2002–Apr</td>
<td>Mozambique</td>
<td>O1 El Tor Ogawa</td>
<td>Cm (57.9), Co (96.6), Tet (97.3), Qu (4.2)</td>
<td>ND</td>
<td>Mandomando <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>2004</td>
<td>Thua Thien,</td>
<td>O1</td>
<td>Amo, Ery</td>
<td>ICE</td>
<td>Bani <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>Vietnam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>ladesh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>Hangzhou, East</td>
<td>O139</td>
<td>Amp, Sm, Gent, Tet, Cm, SXT</td>
<td>pMRV150; pIP1202-like plasmid (IncA/C plasmid in MDR Y. pestis)</td>
<td>Pan <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>2004</td>
<td>Chennai, India</td>
<td>O1 El Tor Ogawa (classical CTXΦ)</td>
<td>Co, NA, nitrofurantoin, Spec, Sm, SXT</td>
<td>Class I integron SXT element</td>
<td>Goel <em>et al.</em> (2010)</td>
</tr>
</tbody>
</table>
ABC (ATP-binding cassette) multidrug resistance efflux pump. VcaM confers resistance to structurally divergent drugs (e.g. tetracycline, norfloxacin, ciprofloxacin and doxorubicin).

*V. cholerae* uses an array of MATE-family efflux systems, namely VcmB, VcmD, VcmH, VcmN, VcmA and VcrM (Begum *et al.*, 2005; Huda *et al.*, 2003). In addition, the *V. cholerae* O1 El Tor N16961 genome carries a homologue of NorM in *Vibrio parahaemolyticus* (Heidelberg *et al.*, 2000) that mediates resistance to hydrophilic fluoroquinolones, aminoglycosides and norfloxacin (Morita *et al.*, 1998; Singh *et al.*, 2006).

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). It was 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.*, 2009). The EmrD-3 gene is also present in the genome of the El Tor strain N16961 and the non-O1/non-O139 strains V51, V52 and TMA 21 (M. Kitaoka, unpublished observation).

The *V. cholerae* RND efflux systems are encoded by six operons (*vexRAB*, *vexCD*, *vexEF*, *vexGH*, *vexIJK* and *vexLM*) (Bina *et al.*, 2006, 2008) and exhibit particularly broad substrate specificity (Van Bambeke *et al.*, 2003).

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Strain</th>
<th>Antibiotic resistance</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004–2006</td>
<td>Iran</td>
<td>ND</td>
<td>SXT, Sm, Cm</td>
<td>SXT element</td>
<td>Adabi <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>Oct 2004–Mar 2006</td>
<td>Senegal</td>
<td>O1 El Tor</td>
<td>Co (90.3)</td>
<td>ND</td>
<td>Manga <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>2004–2005</td>
<td>Cameroon</td>
<td>O1</td>
<td>SXT (100), Amp</td>
<td>ND</td>
<td>Ngandjio <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>2005</td>
<td>Iran</td>
<td>O1 El Tor Inaba</td>
<td>Nf (97), Cpr (92), Kan (88), amikacin (85), Tet (77), Dox (67), Fz (100), SXT (98), Ery (62)</td>
<td>ND</td>
<td>Keramat <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>Aug 2006–Sep 2008</td>
<td>North-west Ethiopia</td>
<td>O1 Inaba</td>
<td>Co (100), Cm (94), Amp (89), Ery (15), Tet (6.2), Cpr (1.2)</td>
<td>ND</td>
<td>Ahera <em>et al.</em> (2010)</td>
</tr>
<tr>
<td>2006</td>
<td>Accra, Ghana</td>
<td>O1</td>
<td>SXT</td>
<td>SXT element (88.9)</td>
<td>Class 2 integron (81.5)</td>
</tr>
<tr>
<td>Dec 2006–Feb 2007</td>
<td>Namibia</td>
<td>O1 El Tor Inaba</td>
<td>SXT, Sm</td>
<td>ND</td>
<td>Smith <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>Aug–Sep 2007</td>
<td>India</td>
<td>O1 El Tor</td>
<td>Amp, co-amoxiclav, aztreonam, Co, Ery, metronidazole, NA, Neo, nitrofurantoin, oxacillin, PB, Spe, Sm, Tri, Vanc</td>
<td>Class 1 integron SXT element</td>
<td>Jain <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>2008</td>
<td>Iran</td>
<td>O1 El Tor Inaba</td>
<td>Inaba: NA (100), Amo (100), SXT (95.7), Fz (91.3) NAG: Ery (77.4)</td>
<td>ND</td>
<td>Ranjabar <em>et al.</em> (2010)</td>
</tr>
<tr>
<td>Jun 2008–Jan 2009</td>
<td>Nepal</td>
<td>O1 El Tor Ogawa</td>
<td>Fz (100), NA, Co</td>
<td>ND</td>
<td>Karki <em>et al.</em> (2010)</td>
</tr>
<tr>
<td>Jan 2009</td>
<td>Zimbabwe</td>
<td>O1 El Tor Ogawa and Inaba</td>
<td>Fz, SXT</td>
<td>ND</td>
<td>Islam <em>et al.</em> (2009)</td>
</tr>
</tbody>
</table>
Interestingly, the *V. cholerae* RND systems play a role not only in the efflux of a variety of compounds (e.g. Triton X-100, SDS, polymyxin B, erythromycin, bile salts, penicillin), but also in colonization (Bina et al., 2008). Collectively, these results indicate that efflux pumps are not employed exclusively for drug resistance, but also play a role in the expression of important virulence genes in *V. cholerae*.

**Spontaneous mutations**

Resistance to antimicrobial compounds can arise through spontaneous mutations in the bacterial chromosome. Mutations conferring resistance to the cell wall biosynthesis inhibitor alafosfalin and to the DNA replication inhibitor family of quinolones are well documented in *V. cholerae* (Allen et al., 1979; Gellert et al., 1977; Goss et al., 1965; Sugino et al., 1977). A comprehensive study during the 1980 cholera epidemic in the United Republic of Tanzania (Towner et al., 1980) revealed that *V. cholerae* genes undergo higher mutation rates than *E. coli* genes, facilitating resistance to antibiotics such as alafosfalin (Atherton et al., 1979). Studies with radiolabelled alafosfalin demonstrated that the mechanism of resistance involved impaired uptake of the drug; however, the chromosomal mutation leading to this resistance remains unknown (Towner et al., 1980). In 2002, Baranwal and coworkers reported an increase in quinolone resistance in *V. cholerae* clinical isolates and conducted a study to determine the mechanism behind this resistance (Baranwal et al., 2002). They found chromosomal mutations in the genes gyrA and parC, which encode subunits of DNA gyrase and topoisomerase IV, respectively. Presumably these mutations alter the affinity of DNA gyrase and topoisomerase IV for the antibiotic, and thus protect *V. cholerae* from quinolones. In 2010, Kim and coworkers conducted a retrospective study to determine mechanisms accounting for the increasing quinolone resistance observed in Dhaka Hospital in Bangladesh over a 6-year period from 2002 to 2008. Similar to the study by Baranwal et al. (2002), they found that *V. cholerae* had accumulated chromosomal mutations in the genes gyrA and parC, conferring increased resistance to quinolones (Kim et al., 2010). It was noted that strains collected earlier in the 6-year period contained a mutation only in gyrA and, over time, these strains gained the additional mutation in parC, conferring higher levels of quinolone resistance. It appears that subjection of *V. cholerae* to quinolones during cholera treatments engendered increasing resistance to these antibiotics. A multitude of studies (Abera et al., 2010; Das et al., 2008; İslam et al., 2009; Karki et al., 2010; Ngandjio et al., 2009; Ranjarbar et al., 2010; Roychowdhury et al., 2008) have documented resistance of *V. cholerae* towards a variety of antibiotics commonly used to treat cholera (e.g. tetracycline, erythromycin, chloramphenicol, quinolones); however, these studies were designed to evaluate the antibiotic resistance of *V. cholerae* strains and not to elucidate mechanisms of resistance. As such, it is possible that spontaneous chromosomal mutations are responsible for resistance to any of the above named antimicrobial compounds.

**SXT elements and integrons**

The spread of antibiotic-resistant *V. cholerae* is also facilitated by horizontal gene transfer via self-transmissible mobile genetic elements, including SXT elements – mobile DNA elements belonging to the class of integrative conjugating elements (ICEs). The SXT element was first described in *V. cholerae* serogroup O139 based on its ability to harbour genes that provide the host bacterium with resistance to sulfamethoxazole, trimethoprim and streptomycin (Waldor et al., 1996). Today, many strains of the O1 and O139 serogroups isolated around the globe have acquired SXT elements through natural spread (Hochhut et al., 2001; Burrus et al., 2006). Similar to conjugative plasmids, ICEs are exchanged between two bacteria by conjugation; however, unlike plasmids, ICEs need to integrate into the chromosome to propagate since they do not have the capacity to replicate autonomously (Hochhut & Waldor, 1999). SXT elements integrate into the 5′-end of *prfC*, a chromosomal gene that encodes peptide chain release factor 3 (Hochhut & Waldor, 1999). Site-specific recombination between a segment of the circular ICE (*attP*) and the nearly identical chromosomal sequence (*attB*) is mediated by the SXT-encoded (integrase family) tyrosine recombinase Int (Hochhut & Waldor, 1999). Once integrated, the SXT element replicates with the host chromosome. Subsequent conjugation with other bacteria is enabled by interbacterial translocation through an apparatus encoded by genes in the SXT element that mediates transfer to a wide range of bacterial species, including clinical isolates of *Providencia alcalifaciens* (reviewed by Burrus et al., 2006). The SXT element excises from the chromosome with the help of the directionality factor Xis and the integrase Int (Burrus & Waldor, 2003). SXT elements have been found with high frequency in numerous organisms since their discovery in 1996 (Waldor et al., 1996). This is most likely the result of dispersal by horizontal gene transfer. Beaber et al. (2004) found that horizontal dissemination of SXT-encoded antibiotic resistance genes is regulated by the bacterial SOS response. These investigators elegantly demonstrated that stress alleviates the SXT-encoded repressor SetR, which in turn activates excision and conjugation of the element. Interestingly, ciprofloxacin acts as an inducing molecule, thus promoting horizontal transfer of SXT elements. This work suggests that antimicrobial agents can promote the spread of antibiotic resistance genes, contributing to the successful spread of SXT elements in the *V. cholerae* population. Despite their rapid spread, SXT elements are unstable and undergo rapid change. For example, an SXT element from a Laos *V. cholerae* isolate lost resistance to trimethoprim and gained genes encoding a putative exonuclease and helicase (Iwanaga et al., 2004). Besides conferring antibiotic resistance, SXT
elements have the capacity to mobilize conjugative plasmids and genomic islands in trans (Daccord et al., 2010; Hochhut et al., 2000), providing alternative mechanisms for antibiotic resistance gene transfer.

Dissemination of antibiotic resistance genes is also facilitated when *V. cholerae* cells share mobile integrons with other bacterial cells. Integrons are genetic assembly platforms that incorporate exogenous open reading frames such as antibiotic resistance cassettes via site-specific recombination in a site proximal to a promoter that drives their expression. All *V. cholerae* isolates harbour large chromosomal integrons, giving them the capacity to rapidly transfer gene cassettes containing antibiotic resistance genes (Mazel, 2006). In addition, clinical and environmental *V. cholerae* can also contain mobile integrons, which are smaller (0–10 cassettes), but are embedded within mobile elements such as conjugative plasmids and transposons (Mazel, 2006) and can disseminate horizontally. Class 1 integrons are by far the most frequent type in this category and are closely associated with a Tn402 transposon, whereas class 2 integrons are associated with a Tn21 transposon. Both classes carry multiple gene cassettes encoding antibiotic resistance genes, such as *dfrA1* (trimethoprim resistance) (Dalsgaard et al., 1999; Opintan et al., 2008). The recombination activity of integrons (like SXT elements) is stimulated by the SOS response, which can be triggered by various antibiotics (Beaber et al., 2004; Guerin et al., 2009).

**Conjugative plasmids**

Because tetracyclines are oral antibiotics often given to patients during rehydration therapy (Greenough et al., 1964), it is not surprising that many *V. cholerae* strains resistant to these drugs have been identified. One of the first reported tetracycline-resistant strains (exhibiting resistance to streptomycin, tetracycline and chloramphenicol) was isolated in the Astrakhan region of the USSR circa 1970 (Fig. 1). The resistance was transferable to *E. coli* K-12, and the strain was shown to carry a single plasmid (Hedges & Jacob, 1975). Similarly, a cholera outbreak in Matlab, Bangladesh, in 1979 was caused by a strain that carried a multiple-drug-resistant plasmid transferable through conjugation with other bacteria, including *E. coli* (Glass et al., 1983). This plasmid conferred resistance to a number of antibiotics in addition to tetracycline, including ampicillin, kanamycin, streptomycin, gentamicin and trimethoprim. However, this multiple-drug-resistant strain disappeared from the area within a decade (Faruque et al., 1998). Table 3 includes *V. cholerae* strains that have developed episomally propagated antibiotic resistance.

**Conclusions**

Antibiotics are often used in combination with rehydration therapy because they are believed to relieve the symptoms of cholera faster than rehydration treatment alone, and because a shorter disease duration lessens the transmission of infectious *V. cholerae*. Because antibiotics are widely used as part of the cholera treatment regimen, the number of pathogenic *V. cholerae* strains resistant to one or more antibiotics is increasing, as summarized in Table 3. As an environmental organism, *V. cholerae* has the means to acquire resistance genes from the natural and human gut, *V. cholerae* may share these resistance traits with commensals or other enteric pathogens, thereby complicating the treatment of an array of infections. To prevent the spread of resistance, it is crucial to limit the use of antibiotics to cholera patients whose lives cannot be saved by rehydration therapies alone.

These circumstances underline the necessity for alternative strategies and novel approaches in managing this disease. A milestone for effective cholera control will be the development of an inexpensive oral vaccine that protects young children from contracting the disease (WHO, 2010). However, in spite of remarkable recent advances in the understanding of the host–pathogen interaction and molecular mechanisms underlying cholera pathogenesis, such a vaccine has yet to be developed (Provenzano et al., 2006).

Prior to the advent of molecular biology and antibiotics, lytic bacteriophages (vibriophages) were employed to control cholera outbreaks in India in the late 1920s and early 1930s (Summers, 1993). Phage therapy should be revisited as *V. cholerae* populations in the environment and human host are naturally controlled by serogroup-specific bacteriophages (Faruque et al., 2005).

Drugs with antisecretory effects have been tested in clinical trials and some have been shown to limit dehydration in cholera patients; aspirin (Islam et al., 1986), berberine (Rabbani et al., 1987), clonidine (Rabbani et al., 1989) and somatostatin (Molla et al., 1984) did not cause a significant reduction in fluid secretion, while indomethacin and nicotinic acid reduced fluid secretion (Van Loon et al., 1992; Rabbani et al., 1983). Inhibitors of CFTR, the molecular target of cholera toxin, show promise as a treatment option. Glycine hydrazide-based inhibitors inhibit fluid loss in a mouse model (Sonawane et al., 2006), and should be tested in clinical trials.

Another promising therapeutic approach entails the development of drugs that disable the bacterium by inhibiting known virulence mechanisms (Hung et al., 2005). Transcriptional activators ToxR, ToxT and TcpP required for the synthesis of cholera toxin and the TCP represent promising targets. As a guiding example, Hung et al. (2005) identified a small molecule, virstatin, that protects infant mice from intestinal colonization by *V. cholerae* by inhibiting ToxT. Because such drugs render *V. cholerae* harmless without killing the organism, it has been
proposed that the lowered selective pressure reduces the frequency of emerging resistant strains (Clatworthy et al., 2007).

The latest research suggests that V. cholerae has developed strategies to compete with bacterial neighbours (MacIntyre et al., 2010). MacIntyre and colleagues demonstrated that V. cholerae can kill a variety of Gram-negative bacteria including the common intestinal symbiont E. coli. If this competition turns out to be essential for V. cholerae colonization of the small intestine, therapeutic measures could be developed to disrupt the mechanism(s) employed by these pathogens to outcompete members of the intestinal microflora.

Until new treatment options become available, the provision of safe water sources is the most practical and least expensive approach in fighting the transmission of this devastating disease. Safe water can be used to prepare rehydration solutions on site to save the cost of prepackaged solutions; this would allow the inexpensive treatment of many more patients, as prepackaged solutions are often in short supply, and would reduce the need to administer antibiotics.

In settings with poor sanitation, shed V. cholerae reaches the water supply and is transmitted by ingestion of contaminated water. V. cholerae strains that spend a longer portion of their life cycle in water between hosts are postulated to display increased toxigenicity compared to those that are transmitted rapidly between hosts (Ewald, 1994). This hypothesis suggests that a change in transmission mode (i.e. through improved sanitation) favours less toxigenic strains (Ewald, 1994). Further studies are required to determine whether we can manipulate the evolution of V. cholerae towards a more benign, less deadly form of disease.

However, until these intriguing and novel approaches in cholera management materialize, we will have to fall back on three key principles in managing this potentially deadly scourge: clean water supplies, containment of cholera patients to stop transmission, and rehydration treatment with use of antibiotics only under life-threatening circumstances.

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