Proceedings of ICMMGH 2026 Symposium: Biomedical Imaging and AI Applications in Neurorehabilitation
DOI: 10.54254/2753-8818/2026.AU30840

Discussion about Mechanism of Doxycycline and Azithromycin in the Treatment of Cholera

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Abstract. The bacterium Vibrio Cholerae is the pathogen of cholera. The world has experienced seven cholera pandemics since 1817, the seventh of which commenced in 1961 and continues to the present day. According to 2015 data, the annual universal burden of cholera was approximated at 1.3–4 million cases, with fatalities ranging from 21,000 to 143,000. It brings continuous global health threat, particularly in regions with poor sanitation, having limiting access to water. In current cure, semi-synthetic antibiotics play a crucial role in treating disease. This paper discusses two key antibiotics used in cholera treatment—doxycycline and azithromycin—comparing their mechanisms of action, efficacy, and resistance patterns. The antibacterial mechanism of doxycycline, a tetracycline antibiotic, involves the inhibition of protein synthesis. This is achieved through its binding affinity for the 30S subunit of the bacterial ribosome. Azithromycin, a macrolide, acts on the 50S subunit, blocking translation. While both drugs are effective, emerging resistance and side effects are more necessary to be carefully considered in practical use. This discussion highlights the importance of antibiotic stewardship and alternative strategies in cholera management.

Keywords: Cholera, Vibrio cholerae, doxycycline, azithromycin

1. Introduction

Cholera is an acute diarrheal illness resulting from disease with the Gram-negative bacterium Vibrio cholerae, mainly affecting through serogroups O1 and O139. Transmission of the pathogen occurs via the ingestion of contaminated water or food. World Health Organization (WHO) estimates indicate an annual global incidence of 1.3 to 4 million cases, resulting in 21,000 to 143,000 deaths worldwide [1] The hallmark of cholera is serious watery diarrhea, leading to rapid dehydration, metabolic acidosis, and death if the patient is untreated.

As a preventable and treatable condition, cholera can be controlled through the implementation of various strategies. In 2017, the Global Task Force on Cholera Control (GTFCC) set forth an ambitious strategy aimed at eliminating endemic cholera in 20 countries and reducing cholera-related mortality by 90% before the year 2030 [1]. The strategy, entitled "Ending Cholera: A Global Roadmap to 2030," prioritizes key pillars including the reinforcement of public health infrastructure, the enhancement of surveillance systems for early outbreak detection, and the improvement of

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Water, Sanitation, and Hygiene (WASH) conditions. Furthermore, the plan emphasizes expanding access to oral rehydration solutions and increasing vaccination coverage.

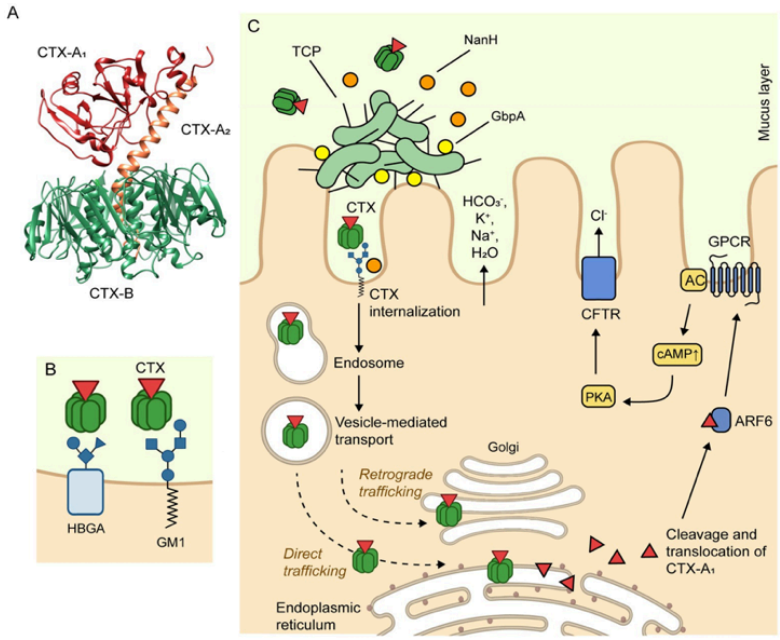

Figure 1. Mechanism of action of Cholera toxin [2]

The mechanism of cholera toxin is shown in figure 1.

(A) CTX is composed of a heterodimeric CTX-A subunit, which consists of two polypeptide chains, CTX-A1 and CTX-A2 with a single disulfide bond between them. The CTX-A2 helical peptide serves as a structural link between the CTX-A1 chain and the pentameric CTX-B subunit, the latter of which is an assembly of five polypeptide chains, all the same.

(B) The pentameric CTX-B subunit mediates toxin uptake into cells by specifically attaching to GM1 gangliosides, its primary receptor, or to histo-blood group antigens (HBGAs), which act as a secondary binding site on intestinal epithelial cells.

(C) Vesicles with toxin are retrograde trafficked to the Golgi body, and then onto the endoplasmic reticulum (ER), where the protein disulfide isomerase (PDI) breaks the bond between CTX-A1 and CTX-A2. Some vesicles move to ER without passing through Golgi body, which is called direct trafficking. Following its translocation into the cytosol from the endoplasmic reticulum, the CTX-A1 subunit binds to ADP-ribosylation factor 6 (ARF6). The resulting CTX-A1/ARF6 complex subsequently activates adenylyl cyclase (AC) via the ADP-ribosylation of a G protein-coupled receptor (GPCR). This activation of AC induces the catalytic alteration of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) continuously increasing the intracellular cAMP concentration. This activates protein kinase A (PKA), phosphorylating the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel proteins, releasing electrolytes and water into the small intestine. It eventually causes the serious diarrhea and dehydration [2].

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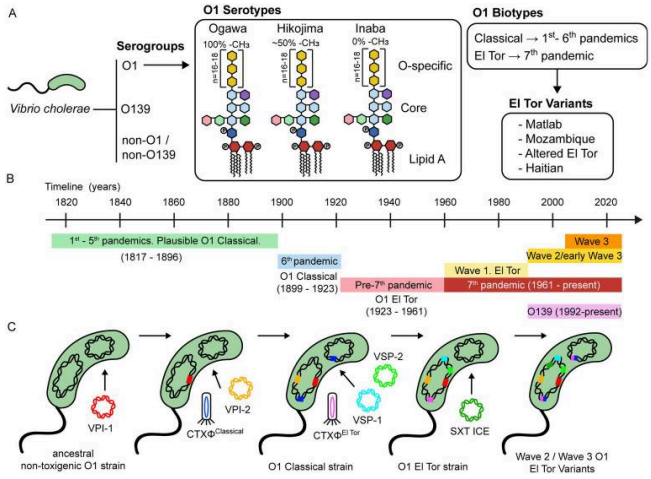

Figure 2. Classification and evolution of V. cholerae [2]

The classification and evolution of V. Cholerae is shown in figure 2.

(A) The classification of Vibrio cholerae is predicated according to the O antigen structure of its lipopolysaccharide (LPS). Serogroup O1 strains are subdivided into three principal serotypes: Ogawa, Hikojima, and Inaba, which are distinguished by the degree of methylation on the terminal perosamine residue, as illustrated. Furthermore, the O1 serogroup is categorized into two major biotypes, Classical and El Tor, defined by distinct phenotypic and genetic markers. In last few decades, surveillance has documented the emergence of variant strains, which possess a combination of genetic features derived from both Classical and El Tor biotypes.

(B) A chronological depiction of the seven recorded cholera pandemics.

(C) A schematic model illustrating the evolutionary mechanisms responsible for the acquisition of virulence in serogroup O1 [2].

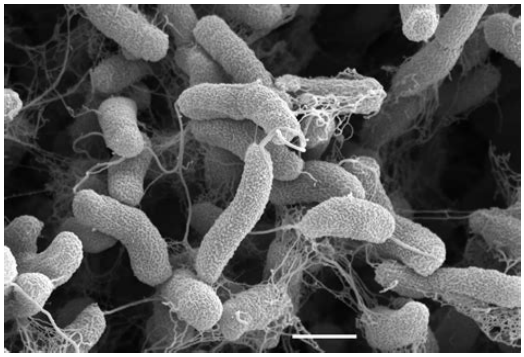

Figure 3. V. Cholerae electron micrograph

The electron micrograph of V. Cholerae is shown in Figure 3.

2. Doxycycline

Doxycycline is a broad-spectrum semi-synthetic tetracycline antibiotic that is used most often in the treatment of cholera. It also demonstrates activity against a wide variety of Gram-positive and Gram-negative bacteria and several protozoan species. The antibacterial action of doxycycline stems from its ability to bind the 30S ribosomal subunit, which interferes with the bacterial protein synthesis machinery.

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2.1. Mechanism of doxycycline

It specifically binds to the A-site (aminoacyl-tRNA binding site), primarily interacting with conserved regions of 16S rRNA. The key nucleotides are G1197 and C1198 on helix 31, U1052 and G1053 on helix 34. In 30S ribosome, doxycycline physically blocks the entry of aminoacyl-tRNA into the A-site, blocking the codon-anticodon pairing.

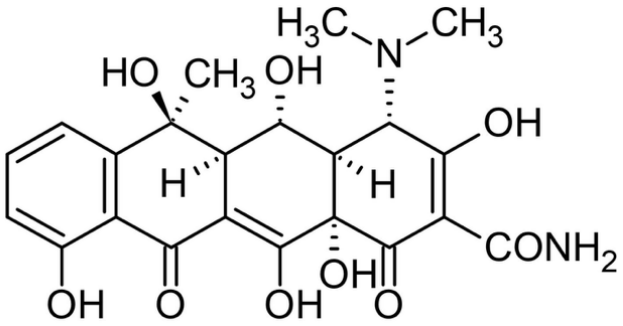

Figure 4. Chemical structure of oxytetracycline

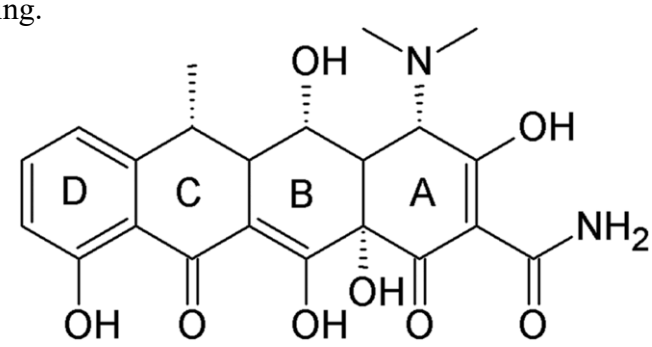

Figure 5. Chemical structure of doxycycline

Chemical structure of oxytetracycline is shown in figure 4, which is a first-generation antibiotic. Chemical structure of doxycycline is shown in figure 5. Doxycycline is synthesized as the oxytetracycline is unstable and its lipophilicity is low. When comparing them, two significant change is the key that changes their chemical properties. Removal of the C6 hydroxyl group (-OH → -H) and removal of a hydrogen at A5. They optimize electrostatic interactions with 16S rRNA and expand half-life of doxycycline to 18 to 22 hours, comparing to oxytetracycline's 6 to 8 hours. This reduces the possibility of increasing resistance, as the dosing frequency is much lower. In addition, the oral bioavailability can be higher than 90%, improving the efficiency of cure.

Emerging resistance has been a problem in treatment using doxycycline. The primary mechanism is Efflux Pumps controlled by Tet(A), Tet(B) and Tet(D), pumping doxycycline out to lower intracellular concentration. Another emerging mechanism is ribosomal protection. Tet(M) RPP binds to the ribosome, displacing doxycycline and keeping the normal translation of mRNA. As shown in Figure 6, the resistant bacteria need much higher concentration of doxycycline to be killed than sensitive bacteria [3].

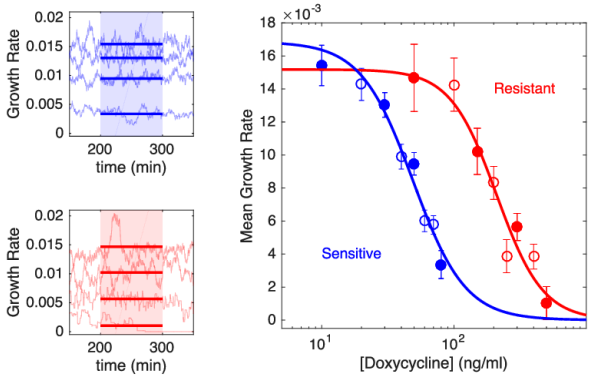

Figure 6. Dose-response curve of doxycycline

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2.2. Experiment

The double-blind clinical trial evaluated the efficacy of doxycycline compared to tetracycline and placebo in treating actively purging cholera patients. Fifty-one bacteriologically confirmed cases were divided into three groups: 17 received doxycycline (2 mg/kg initially, then once daily), 15 received tetracycline hydrochloride (20 mg/kg/day in divided doses every 6 hours), and 19 received a placebo. The results demonstrated that both antibiotic regimens were significantly more effective than placebo, with doxycycline and tetracycline reducing mean stool volume by 48% and 52% (5.1 vs. 4.8 liters vs. placebo's 10.1 liters), intravenous fluid requirements by 41% and 43% (5.7 vs. 5.5 liters vs. placebo's 9.7 liters), and diarrhea duration by 56% and 49% (28 vs. 33 hours vs. placebo's 64 hours), respectively. While tetracycline achieved slightly faster clearance of Vibrio cholerae (1.8 vs. 2.6 days), the clinical outcomes were comparable between the two antibiotics. The study highlighted doxycycline's practical advantages, including less frequent dosing (reducing nursing demands by two-thirds) and safety in patients with potential renal impairment, despite its higher per-dose cost. These findings supported doxycycline as an effective, convenient alternative to tetracycline for cholera treatment, particularly beneficial in epidemic settings with limited healthcare resources. The research also noted that while cholera-associated renal failure had become rare with modern rehydration practices, doxycycline's safety profile made it preferable for high-risk cases with prolonged dehydration [4].

2.3. Clinical use

In clinical use, there are also other considerations. Doxycycline has to be used with ORS to reduce dehydration. For women in pregnancy, doxycycline brings the fetus risk of fetal bone and tooth malformations. For children aging smaller than 8, it may cause permanent tooth staining or enamel hypoplasia. Severe liver disease is also possible when applying this antibiotic. Other common Side effects include GI disturbances, photosensitivity, esophageal irritation and vaginal candidiasis. Therefore, although it’s still the most common treatment with cholera, the increasing resistance and certain people makes it lose effect sometimes.

3. Azithromycin

Azithromycin is a broad-spectrum semi-synthetic antibiotic that is also commonly used in the treatment of cholera. Azithromycin suppresses bacterial growth by attaching to the 50S ribosomal subunit, halting protein production.

3.1. Mechanism of azithromycin

Azithromycin works by impeding bacterial translation through its interaction with the 50S ribosomal subunit. Its binding site is located in the 23S rRNA, particularly within the peptidyl transferase center (PTC), which is essential for peptide bond formation. The key nucleotides involved are A2058 and A2059 in domain V of the 23S rRNA. By binding to this site, azithromycin blocks the elongation of the peptide chain, preventing the transfer of the growing polypeptide from the A-site to the P-site.

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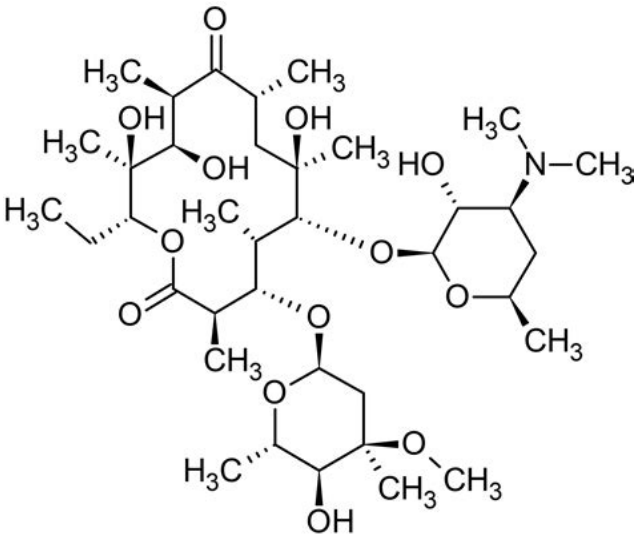

Figure 7. Chemical structure of azithromycin

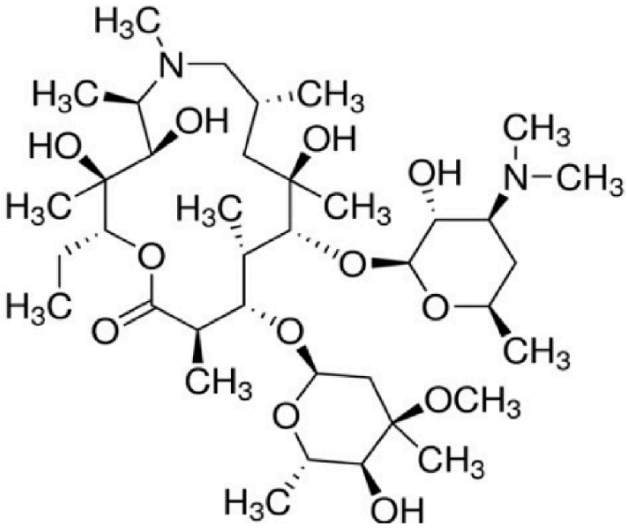

Figure 8. Chemical structure of erythromycin

Figure 7 shows the chemical structure of azithromycin, which is derived from the erythromycin in figure 8. Azithromycin is synthesized as the replacement of oxygen double bond with N-CH3 group makes it more stable in acidic environment, so the patient can be dosed orally. Gastric acid cannot break its structure. Azithromycin optimizes electrostatic interactions with 23S rRNA and expands half-life of azithromycin to approximately 68 hours, decreasing the dosing times and possibility for pathogen to develop resistance. In addition, the oral bioavailability of azithromycin is approximately 38% [5].

Emerging resistance to azithromycin in Vibrio cholerae poses a significant threat to cholera treatment. The data shows that since 1993, the O139 serogroup has shown resistance rates as high as 43% [6]. The primary resistance mechanism involves the up-regulation of efflux pumps, with the RND-family VceB system playing a crucial role in actively expelling azithromycin from pathogens, thereby reducing intracellular drug concentrations. Additionally, the MacAB-TolC efflux system, homologous to E. coli's macrolide-specific pump, contributes to resistance by specifically targeting azithromycin.

3.2. Experiment

The study conducted as a double-blind, randomized trial, evaluated the efficacy of single-dose azithromycin (1g) versus single-dose ciprofloxacin (1g) in treating severe cholera in adults infected with Vibrio cholerae O1 or O139. The results showed that azithromycin was far superior, achieving clinical success (cessation of watery stools within 48 hours) in 73% of patients compared to just 27% with ciprofloxacin, while bacteriologic success (inability to isolate V. cholerae after 48 hours) was observed in 78% of azithromycin-treated patients versus only 10% of those receiving ciprofloxacin. Additionally, patients treated with azithromycin experienced significantly better secondary outcomes, including a shorter median duration of diarrhea (30 hours vs. 78 hours), fewer stools, lower stool volume, and reduced vomiting. The study attributed ciprofloxacin's poor performance to the diminished susceptibility of circulating V. cholerae O1 strains—despite these strains technically meeting standard susceptibility thresholds—suggesting that current resistance criteria for fluoroquinolones may need reevaluation. These findings position azithromycin as an effective single-dose treatment for cholera in adults, complementing prior evidence of its efficacy in

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children. However, the authors caution that emerging resistance to azithromycin in some V. cholerae strains underscores the urgent need for new antimicrobial options, particularly in regions where cholera remains endemic and resistance patterns continue to evolve [7].

3.3. Clinical use

In clinical use, azithromycin is more suitable for pregnant women and young children. People allergic to doxycycline can also use azithromycin. It usually takes 3 to 5 days to cure the patient completely, 7 days for critical patient.

4. Conclusion

In conclusion, as the cholera is still a global health threat, especially because the pathogen mutates quickly, we still need to find the answer of how to treat it with most effective drug. Antibiotics is still the main drug in treatment of cholera, but the lack of large-scale surveillance data in endemic regions leads to the unclear awareness of resistance in latest generation of pathogen. In future, more modification on antibiotics to make it more efficient is necessary. Research on El Tor type pathogen also needs to be going on. We propose that WHO and other agencies should publish more guidelines online, helping with prevention of cholera over the world.

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