



Antibiotic Resistance of Cholera in Hodeidah, Yemen 2017 versus 2024: Experimental Design

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Abstract

Background: Cholera, caused by *Vibrio cholerae* (*V. cholerae*), remains a significant global health threat, particularly in low- and middle-income countries (LMICs) with inadequate sanitation. The increasing emergence of antimicrobial resistance (AMR) in *V. cholerae* strains complicates treatment, as many commonly used antibiotics (e.g., tetracycline, doxycycline, azithromycin) become less effective. This results in treatment failures, higher morbidity and mortality, and more challenging outbreak control. Regions like Yemen reported high AMR prevalence in cholera strains. **Objectives:** Therefore, the study aimed to monitor the effectiveness of the antibiotics that used in treatment of cholera infection based on the inhibition zone measurements. **Methods:** 94 acute watery fecal samples were collected from Diarrhea Treatment Center (DTC) (isolation area) and Center of Tropical Medicine and Infectious Diseases (CTMID) of Authority of Public AL-Thawara Hospital, Hodeidah, Yemen, Outbreak, 2017 and 2024. The *V. cholerae* were isolated and diagnosed based on standard microbiological methods. The resistance of antibiotics was monitored based microbiological assay in vitro. **Results:** The results showed that 34/94 cases (36.17%) of *V. cholerae* were confirmed. The clinical cholera isolates were sensitive for ciprofloxacin, doxycycline, and tetracycline were stable with the time in 2017 versus 2024, while the resistance for azithromycin were recorded partially (50 %) in 2017, and developed into 100 % completely in 2024. **Conclusion:** The study concluded that ciprofloxacin, followed by doxycycline and tetracycline, is most effective against *V. cholerae*. It warns against the continued use of azithromycin due to increasing resistance. The findings call for a revision of cholera treatment protocols, emphasizing the need to discontinue azithromycin and to adopt antibiotics based on local resistance patterns, clinical efficacy, and cost-effectiveness.

Keywords: Cholera, AMR, Yemen

1. Introduction

Cholera, caused by the gram-negative bacterium *Vibrio cholerae* (*V. cholerae*), is a severe diarrheal disease that leads to rapid dehydration and hypovolemia, especially in low- and middle-income countries (LMICs) with poor sanitation, or in areas affected by natural disasters and humanitarian crises. The disease manifests as large-volume, watery diarrhea, and without treatment, the mortality rate can be as high as 50%. However, with prompt rehydration and antibiotic treatment, mortality can be reduced to less than 1%. Despite these advances, cholera remains a significant public health challenge due to increasing endemicity, unpredictable outbreaks, high morbidity and mortality, and rising antimicrobial resistance (AMR). The World Health Organization's (WHO) Global Task Force for Cholera Control has set a target to reduce global cholera cases by 90% and eliminate cholera in at least 20 countries by 2030 [1]. Antimicrobial Resistance in *V. cholerae* (AMR) is a growing concern for global health. The emergence of antibiotic-resistant strains of *V. cholerae* complicates treatment, as many of the antibiotics traditionally used for cholera, such as tetracycline, fluoroquinolones, and azithromycin, are becoming less effective [2,3]. Mechanisms of AMR includes: Efflux Pumps: These pumps actively expel antibiotics out of bacterial cells, lowering the drug's concentration inside the cell and reducing its effectiveness. Genetic Mutations: Chromosomal mutations in *V. cholerae* may lead to resistance by altering the target sites of antibiotics and Mobile Genetic Elements: Resistance genes can be acquired through plasmids, integrons, and Integrative Conjugative Elements (ICE), such as the SXT element, which carries multiple resistance genes *V. cholerae* ability to acquire resistance genes through horizontal gene transfer (HGT) significantly contributes to the spread of AMR [4-7]. AMR in *V. cholerae* poses several challenges: Treatment Fail-

ures: Resistant strains reduce the effectiveness of commonly used antibiotics, leading to prolonged illness, increased health risks, and higher mortality, Epidemic Spread: Resistant strains can spread more widely, complicating efforts to control outbreaks and Epidemic Spread: Resistant strains can spread more widely, complicating efforts to control outbreaks [8,9]. Recent studies have shown that *Vibrio cholerae* strains isolated from different regions are increasingly resistant to common antibiotics like tetracycline, fluoroquinolones, and nalidixic acid. Antibiotic Stewardship and Alternative Therapies Antibiotic; Stewardship The overuse and misuse of antibiotics need to be addressed through stricter guidelines for prescribing antibiotics, especially for non-severe cases Alternative Therapies: Given the rise in AMR, there is a growing interest in alternative strategies, such as using engineered small RNAs (sRNAs) to modulate bacterial gene expression rather than killing the bacteria outright. Research has shown that sRNAs targeting the Type VI Secretion System (T6SS) in *V. cholerae* can effectively reduce its virulence [10]. Access to clean water, proper sanitation, and hygiene (WASH) measures is essential to controlling cholera outbreaks and preventing further spread of AMR [11]. Alternative Antibiotics: Researchers are exploring new antibiotic classes and non-antibiotic therapies to overcome AMR. Reporting of *V. cholerae* strains for antibiotic resistance is critical to understanding the evolving patterns of AMR. The WHO's global action plan for AMR emphasizes the need for improved data collection and monitoring systems, especially in low-resource settings where AMR burdens are highest [2]. Identifying patterns of resistance and tracking the spread of resistant strains can help inform treatment decisions and guide national AMR strategies. In war zones like Yemen, for example, cholera has reached crisis levels due to weak healthcare systems, poor sanitation, and limited access to antibiotics exacerbate the spread

of AMR limited access to healthcare and high malnutrition rates, further complicating treatment and control efforts. Yemen: Amid ongoing war and humanitarian crises, Yemen has experienced one of the largest cholera outbreaks in recent years. Studies have reported a high burden of AMR in cholera cases, with more than 60% of patients admitted to MSF hospitals in Aden showing drug-resistant infections [12]. Therefore, the study aimed to monitor the effectiveness of the antibiotics based on the inhibition zone measurements. On the other mean, the study aimed to monitor the development of antibiotic resistance of cholera

2. Methodology

2.1. Study area, setting and design

The study was carried out in Center of Tropical Medicine and Infectious Diseases (CTMES) - Authority of Public AL Thawara Hospital, Hodeidah, Yemen and National Center of Public Health Laboratories (NCPHL), Hodeidah, Yemen based – laboratory analytical experimental design.

2.2 Sample Collection

Fecal specimens that tested positive for *V. cholerae* O1 and/or O139 through rapid testing were confirmed using traditional culture-based methods. Fresh stool or rectal swab samples were collected from patients with acute diarrhea. Sterile tubes or rectal swabs were utilized to ensure the integrity of the samples. When fresh stool samples were unavailable, rectal swabs were used. During transportation, Cary-Blair medium was employed to maintain bacterial viability. Samples were transported at room temperature if delivered within two hours; otherwise, they were refrigerated at 4-8°C.

2.3 Cholera Cultivation

In the laboratory, enrichment of *V. cholerae* was achieved using Alkaline Peptone Water (APW), incubated at 35-37°C for 6-8 hours. The enriched samples were then inoculated on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar to identify yellow colonies indicative of sucrose fermentation. Biochemical tests confirmed the bacteria as sucrose-fermenting and oxidase-positive. The urease test was negative, the indole test was positive, and the bacteria demonstrated motility but could not utilize citrate. Serotyping with specific antisera identified sero-groups O1 and O139 via visible clumping in the agglutination test.

2.4. Antibiotic Sensitivity

Antibiotic susceptibility testing was performed using Mueller-Hinton agar. Antibiotic discs, including tetracycline, doxycycline, ciprofloxacin, and azithromycin, were placed on the agar to measure zones of inhibition.

2.5 Data Analysis

Data from the study were analyzed to assess the antibiotic resistance patterns of *V. cholerae*. The data obtained were evaluated using standard statistical methods.

3. Results

3.1. Patient characteristics

Watery fecal samples were collected from 94 suspected cases with *V. cholerae* from 8 districts in Hodeidah, Yemen, Outbreak, 2017 aged between 2 and 70 years, including 39 males (41.48%) and 55 females (58.51%). 34/94 cases (36.17%) of *V. cholerae* were diagnosed and confirmed with cholera. The *V. cholerae* were isolated and diagnosed based on standard microbiological methods.

3.2. Antibiotic resistance and sensitivity

The effectiveness of certain antibiotics against isolated *V. cholerae* was evaluated, with the following results: ciprofloxacin: It showed good effectiveness against *V. cholerae*, with an average inhibition zone of 23 ± 3 mm. Doxycycline was less effective

compared to ciprofloxacin, with an inhibition zone of 20 ± 2 mm. Tetracycline showed similar results to doxycycline, with an inhibition zone of 19 ± 2 mm. As for azithromycin, complete resistance was observed in 50% of the samples, while the remaining 50% were sensitive, with an inhibition zone of 18 ± 1 mm. These results indicate that ciprofloxacin was the most effective in combating *V. cholerae*, followed by doxycycline and tetracycline in terms of effectiveness. The results for azithromycin were concerning, as resistance was noted in half of the samples, reflecting the challenges current treatments may face in addressing cholera, especially with the development of antibiotic resistance. The results showed that the resistance was, partly 50 % for azithromycin in 2017 and developed into 100 % completely in 2024. While the sensitivity of cholera for ciprofloxacin, doxycycline and tetracycline were stable with the time (Table 1, Figure 1 and Figure 2).

Table 1 : Demographic data of cholera patients (N : 94)

variables	Number (n)	Percentage (%)
Sex		
• Male	39	41.48
• Female	55	58.51
Age (years)		
• ≤ 5	4	4.25
• 6 - 17	25	26.59
• 18 – 50	54	57.44
• > 50	11	11.70
Residency		
• Urban	82	87.23
• Rural	12	12.76

Table 2 : Resistance and sensitivity data of antibiotics of cholera (N:34)

Antibiotic	2017	2024	z – Test	p – value
	Mean $\pm$ SD	Mean $\pm$ SD		
Doxycycline *	19 $\pm$ 2	20 $\pm$ 2	2.08	0.038
Tetracycline	18 $\pm$ 2	19 $\pm$ 2	2.08	0.038
Ciprofloxacin	22 $\pm$ 2	23 $\pm$ 3	1.38	0.168
Azithromycin **	18 $\pm$ 1	0	NA	0.00

* : Sensitive

** : Resistance

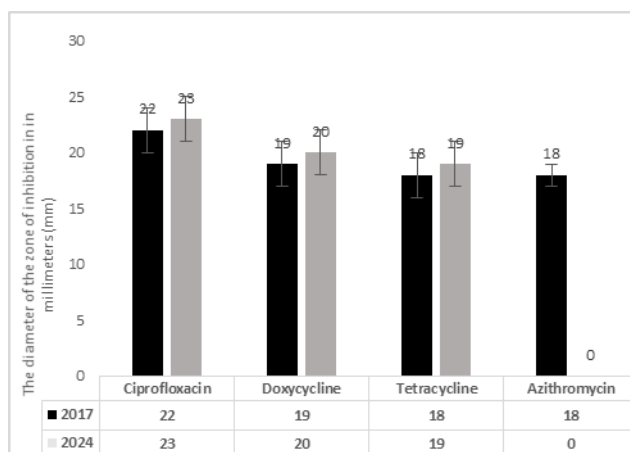


Figure (1): Monitoring of Antibiotic Resistance of *V. cholerae* 2017 versus 2024

4. Discussion

Cholera remains a significant global health threat [11]. Around one billion people lack access to safe water and sanitation, making them vulnerable to cholera. Each year, 3–5 million cholera cases occur, causing 100,000–130,000 deaths, primarily in Africa and Asia. Environmental factors, such as changes in water temperature and nutrient discharge, contribute to the spread of cholera by promoting the growth of phytoplankton, zooplank-

ton, and *V. cholerae*. Cholera rates also rise during floods. Current control strategies have not been very effective in high-burden regions [13]. Progressive renal impairment is a severe complication of Asiatic cholera, often leading to fatal outcomes. In 1921, Rogers linked anuria in cholera to decreased blood pressure and impaired kidney circulation. Later, researchers like Tomb and Wilkinson compared renal changes in cholera to those seen in renal anoxia. In nonfatal cases, prolonged oliguria is followed by a diuretic phase, typical of recovery from acute tubular necrosis. Renal ischemia, caused by shock or hemo concentration, is central to this process. Prompt fluid therapy is crucial to restore renal blood flow and prevent or reduce acute tubular necrosis severity. Potassium depletion, due to diarrhea and poor nutrition, contributes to renal damage, leading to vacuolar changes in tubular cells [14]. While antibiotics alone are ineffective, a combination of antibiotics and fluid replacement can help manage the disease. However, rising antibiotic resistance in *V. cholerae*, including to tetracycline and β -lactams, poses challenges. Recent studies show increasing resistance to tetracycline (50%) and doxycycline (28%) in *V. cholerae* O1 strains. Resistance patterns vary by region, with higher resistance rates in developing countries compared to developed ones. Resistance to antibiotics like amoxicillin and penicillin has decreased over time due to reduced use, while resistance to others like ciprofloxacin has grown. These trends highlight the need for careful antibiotic regulation and continuous monitoring to effectively treat cholera [11]. The importance of understanding antibiotic resistance patterns to better control and treat cholera. The other study found resistance ciprofloxacin (5%) and tetracycline resistance decreased over time, but remained present in 13% of isolates. The resistance to fluoroquinolones like ciprofloxacin increased over the years, especially in Asia. Overall, this analysis emphasizes the need for ongoing surveillance and new treatment strategies to combat rising antibiotic resistance in *V. cholerae* [15]. A meta-analysis of 21 studies, mostly from Asia (n=102), analyzed AMR patterns in *V. cholerae* O1/O139 isolates, covering 648 samples. The weighted pooled resistance (WPR) rates were as follows: azithromycin 1%, ciprofloxacin 3%, doxycycline 7%, and tetracycline 20%. Resistance to ciprofloxacin and tetracycline increased from 1980 to 2020, with significant reductions in doxycycline and ciprofloxacin resistance between 2011–2020 compared to 2001–2010. WHO reported that 2.9 million annual cholera cases, with multi-drug resistant strains posing challenges, especially in low-income countries [16].

Finally, our results agreed with the national guideline of ministry of public health and population (MOPHP) of Yemen for acute watery diarrhea (AWD) control (cholera) of 2024 to avoid the using of azithromycin and erythromycin in treatment of cholera [17], compared with guideline of 2017 where was prescribed the azithromycin and erythromycin in treatment of cholera [18].

5. Conclusion

The study concluded that ciprofloxacin, followed doxycycline and tetracycline is the most effective against *V. cholerae*, while the use of azithromycin may need careful reconsideration due to the spread of resistance. Intensifying research efforts to develop new strategies to combat cholera is crucial in light of the growing issue of antibiotic resistance. Our study highlights a critical revision in antibiotic treatment protocols for *V. cholerae*. Specifically, azithromycin is no longer recommended due to emerging resistance patterns. This change is essential to improve treatment efficacy and to support broader antimicrobial stewardship efforts. The study recommends the use of doxycycline, tetracycline, and ciprofloxacin for the treatment of cholera. These antibiotics were selected based on their proven clinical efficacy, broad-spectrum activity, and cost-effectiveness. The study also highlights the urgency of intensifying research to address the growing threat of antibiotic resistance and support antimicrobial stewardship.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was conducted during our work at CTMID – Al Thawara Public Hospital Authority, Hodeidah, Yemen.

Ethical Approval

The studies involving human participants were reviewed and approved by Ethics Committee of Tropical Medicine and Epidemiology Studies of Hodeidah University (CTMES-HU) Hodeidah, Yemen.

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References

- Ojeda Rodriguez, J. A., Hashmi, M. F., & Kahwaji, C. I. (2024). *Vibrio cholerae* Infection. In *StatPearls*. StatPearls Publishing. Copy <https://www.ncbi.nlm.nih.gov/books/NBK526099/>
- Bertagnolio, S., Dobrev, Z., Centner, C. M., Olaru, I. D., Donà, D., Burzo, S., Huttner, B. D., Chaillon, A., Gebreselassie, N., Wi, T., Hasso-Agopsowicz, M., Allegranzi, B., Sati, H., Ivanovska, V., Kothari, K. U., Balkhy, H. H., Cassini, A., Hamers, R. L., Weezenbeek, K. V., & WHO Research Agenda for AMR in Human Health Collaborators (2024). WHO global research priorities for antimicrobial resistance in human health. *The Lancet. Microbe*, 5(11), 100902. [https://doi.org/10.1016/S2666-5247\(24\)00134-4](https://doi.org/10.1016/S2666-5247(24)00134-4)
- Das, B., Verma, J., Kumar, P., Ghosh, A., & Ramamurthy, T. (2020). Antibiotic resistance in *Vibrio cholerae*: Understanding the ecology of resistance genes and mechanisms. *Vaccine*, 38 Suppl 1, A83–A92. <https://doi.org/10.1016/j.vaccine.2019.06.031>
- Gladkikh, A. S., Feranchuk, S. I., Ponomareva, A. S., Bochalgin, N. O., & Mironova, L. V. (2020). Antibiotic resistance in *Vibrio cholerae* El Tor strains isolated during cholera complications in Siberia and the Far East of Russia. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases*, 78, 104096. <https://doi.org/10.1016/j.meegid.2019.104096>
- Gladkikh, A. S., Feranchuk, S. I., Ponomareva, A. S., Bochalgin, N. O., & Mironova, L. V. (2020). Antibiotic resistance in *Vibrio cholerae* El Tor strains isolated during cholera complications in Siberia and the Far East of Russia. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases*, 78, 104096. <https://doi.org/10.1016/j.meegid.2019.104096>
- Verma, J., Bag, S., Saha, B., Kumar, P., Ghosh, T. S., Dayal, M., Senapati, T., Mehra, S., Dey, P., Desigamani, A., Kumar, D., Rana, P., Kumar, B., Maiti, T. K., Sharma, N. C., Bhadra, R. K., Mutreja, A., Nair, G. B., Ramamurthy, T., & Das, B. (2019). Genomic plasticity associated with antimicrobial resistance in *Vibrio cholerae*. *Proceedings of the National Academy of Sciences of the United States of America*, 116(13), 6226–6231. <https://doi.org/10.1073/pnas.1900141116>
- Kitaoka, M., Miyata, S. T., Unterwieser, D., & Pukatzki, S. (2011). Antibiotic resistance mechanisms of *Vibrio cholerae*. *Journal of medical microbiology*, 60(Pt 4), 397–407. <https://doi.org/10.1099/jmm.0.023051-0>
- Das, B., Verma, J., Kumar, P., Ghosh, A., & Ramamurthy, T. (2020). Antibiotic resistance in *Vibrio cholerae*: Understanding the ecology of resistance genes and mechanisms. *Vaccine*, 38 Suppl 1, A83–A92. <https://doi.org/10.1016/j.vaccine.2019.06.031>
- Chatterjee, P., Kanungo, S., Bhattacharya, S. K., & Dutta, S. (2020). Mapping cholera outbreaks and antibiotic resistant *Vibrio cholerae* in India: An assessment of existing data and a scoping review of the literature. *Vaccine*, 38 Suppl 1, A93–A104. <https://doi.org/10.1016/j.vaccine.2019.12.003>
- Dong, C., Zhang, X., Bao, C., Zhu, Y., Zhuang, L., Tan, Z., Qian, H., & Tang, F. (2015). *Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]*, 49(2), 128–131.
- Nateghizad, H., Sajadi, R., Shivaee, A., Shirazi, O., Sharifian, M., Tadi, D. A., & Amini, K. (2023). Resistance of *Vibrio cholerae* to antibiotics that inhibit cell wall synthesis: A systematic review and meta-analysis. *Frontiers in pharmacology*, 14, 1027277. <https://doi.org/10.3389/fphar.2023.1027277>

12. Verma, J., Bag, S., Saha, B., Kumar, P., Ghosh, T. S., Dayal, M., Senapati, T., Mehra, S., Dey, P., Desigamani, A., Kumar, D., Rana, P., Kumar, B., Maiti, T. K., Sharma, N. C., Bhadra, R. K., Mutreja, A., Nair, G. B., Ramamurthy, T., & Das, B. (2019). Genomic plasticity associated with antimicrobial resistance in *Vibrio cholerae*. *Proceedings of the National Academy of Sciences of the United States of America*, 116(13), 6226–6231. <https://doi.org/10.1073/pnas.1900141116>
13. Al Kamarany, M. ., & Ghouth, A. S. B. (2024). The Ice Berge of Cholera in Hodeidah , Yemen : The Contact Exposure. *Studies in Medical and Health Sciences*, 1(1), 32–35. <https://doi.org/10.48185/smhs.v1i1.1169>
14. Al Sheebani, S.; Al-Kamarany, M.A.; Ghouth, A.B.; Kamal, A.; Alaq, M . .(2018). Acute renal failure induced by cholera: Outbreak of Hodeidah, Yemen, 2017: *European Journal of Pharmaceutical and Medical Research* , 5(8), 188–192. https://www.ejpmr.com/home/abstract_id/4065
15. Wu, Q., Vaziri, A. Z., Omid, N., Hassan Kaviar, V., Maleki, A., Khadivar, P., & Kouhsari, E. (2023). Antimicrobial resistance among clinical *Vibrio cholerae* non-O1/non-O139 isolates: systematic review and meta-analysis. *Pathogens and global health*, 117(3), 235–244. <https://doi.org/10.1080/20477724.2022.2114620>
16. Wu, Q., Vaziri, A. Z., Omid, N., Hassan Kaviar, V., Maleki, A., Khadivar, P., & Kouhsari, E. (2023). Antimicrobial resistance among clinical *Vibrio cholerae* non-O1/non-O139 isolates: systematic review and meta-analysis. *Pathogens and global health*, 117(3), 235–244. <https://doi.org/10.1080/20477724.2022.2114620>
17. **National Guideline for Acute Watery Dairrhea Control (Cholera)** (2024). Ministry of Public Health and Population , Yemen
18. **National Guideline for Acute Watery Dairrhea Control (Cholera)** (2017). Ministry of Public Health and Population , Yemen