Trends of Multidrug Resistance Phenotypes in *Vibrio cholerae* causing Cholera in Bangladesh

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelors of Science in Microbiology

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Approval

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Abstract

Antibiotic resistance is a growing global concern, posing significant challenges in the treatment of bacterial infections. *Vibrio cholerae*, a gram-negative bacterium responsible for the severe diarrheal disease cholera, has exhibited multidrug resistance, limiting the effectiveness of available treatment options. This study aimed to investigate the antibiotic resistance profile of *Vibrio cholerae* strains which were collected from each month between January-September from the year 2022 including isolates associated with a small outbreak occurring in April, 2022. All the strains (n=30) used in this study were *Vibrio cholerae* serogroup O1 and El Tor biotype. The antimicrobial susceptibility tests confirmed that all the test isolated strains were sensitive to Fluoroquinolone, Cephalosporin and Macrolides classes of antibiotics. But alarmingly, there were resistance to Imipenem (IPM), Trimethoprim sulfamethoxazole (SXT), Ampicillin (AMP) and Amoxicillin-clavulanic acid (AMC) with 83% of the strains being multidrug resistant (MDR). The isolated *Vibrio cholerae* strains showed the most significant resistance to AMP and SXT. Antibiotic resistance trend was found to correlate to the prevalence pattern of serotypes, while 94% of the Ogawa serotype was resistant to IPM whereas only 64% of the Inaba strains were resistant to that. The findings from this study will contribute to better treatment strategies for cholera patients, considering the antibiotic resistance profile of *Vibrio cholerae* strains.

Keywords: Antibiotic resistance profile, *Vibrio cholerae,* MDR. Bacterial infections, Cholera, Public Health.

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List of Acronyms

ICDDR,B	International Centre for Diarrhoeal Disease Research, Bangladesh
AMR	Antibiotic resistance
MDRO	Multidrug resistant organism
LSP	Lipopolysaccharide
GalNAc	N-acetylgalactosamine
GlcNAc	N-acetylglucosamine
TcpA	Toxin co-regulated pilin A
CL	Classical
ET	El Tor
TCBS	Thiosulfate citrate bile salts sucrose agar
TTGA	Taurocholate tellurite gelatin agar
CLSI	Clinical and Laboratory Standards Institute
MDR	Multidrug Resistant

Trends of Multidrug Resistance Phenotypes in *Vibrio cholerae* causing Cholera in Bangladesh

1. Introduction

Antibiotics are medicines that treat infections and diseases caused by bacteria through either killing the bacteria or limiting or completely preventing them from multiplying. Antibiotics belong to a larger group of medicines known as "antimicrobials". Antimicrobials include antifungals, antiparasitics, antivirals and antibiotics. The majority of antibiotics function generally by (i) interrupting essential cellular metabolic pathways, (ii) damaging the integrity of cell membranes, (iii) inhibiting the production of DNA, RNA, and proteins and (iv) inhibiting the production or assembly of cell walls [13]. The first synthetic antibiotic discovered was "Salvarsan" which was in 1910.[1],[4] With the discovery of the antibiotic "Penicillin" in 1928 by Alexander Flaming, the golden age of natural product antibiotic began which peaked around the mid-1950s [1],[2]. Since then, a lot of antibiotics have been discovered which enabled the evolution of drug resistance by bacteria. Penicillin resistance was first identified around the year 1940 [1],[5]. The event of a bacteria changing in response to the use of antibiotics by being able to survive or grow despite the presence of antibiotics is termed as antibiotic resistance. Antibiotics resistance makes it hard or impossible to treat bacterial infections. It can lead to more severe illnesses, longer hospital stays, higher medical costs, and more deaths . Antibiotic resistance (AMR) can affect anyone irrespective of age and can be spread between people and animals. Several factors can be responsible for AMR which includes misuse and overuse of antibiotics in humans and animals, the lack of access to quality health care and sanitation, the poor infection prevention and control practices in health care settings, and the environmental contamination by antibiotics and resistant bacteria etc.[3] Bacteria can develop resistance by different mechanisms, for example; by modifying their cell walls or membranes to prevent the entry of antibiotics, producing enzymes that destroy or deactivate antibiotics, altering the targets of antibiotics to reduce their binding or effectiveness, or pumping out antibiotics from their cells using efflux pumps [6],[13]. Bacteria can also acquire resistance genes from other bacteria through horizontal gene transfer mediated by mobile genetic elements such as plasmids, transposons, integrons, or bacteriophages [7]. Gram-negative bacteria have achieved this resistance [9].

According to Breijyeh et al.(2020), Gram-negative bacteria are more resistant than Gram-positive bacteria, and cause significant morbidity and mortality worldwide.

Vibrio cholerae, a gram negative bacteria belonging to the family *Vibrionaceae,* is comma shaped and is responsible for the disease cholera which is severe diarrheal disease that can be deathly if it's not treated. Cholera spreads through the consumption of contaminated food or water containing the bacterium. The primary virulence factor of Vibrio cholerae is the cholera toxin (CT), which is an AB5 toxin comprising one A subunit and five B subunits. The B subunits specifically attach to GM1 gangliosides found on the surface of intestinal cells, facilitating the entry of the A subunit into the cells [34]. Once inside, the A subunit activates adenylate cyclase, an enzyme that generates cyclic AMP (cAMP), a signaling molecule that disrupts the normal transportation of electrolytes and water across the cell membrane. As a result, there is an extensive release of fluid and electrolytes into the intestinal lumen, leading to profuse watery diarrhea and dehydration. The mode of transmission of this organism is water. Vibrio spp. can thrive in many aquatic habitats, including freshwater, estuarine, and marine ecosystems. *V. Cholerae*, specifically its O1 and O139 serogroups, is responsible for 7 global epidemics [10],[13]. There are more than 200 serogroups of *Vibrio cholerae*. Serogroups are defined by the O antigen of the lipopolysaccharide (LPS), which is a molecule on the outer membrane of the bacterium. Within the O1 serogroup, there are also two major biotypes, classical and El Tor, which differ by their biochemical and genetic characteristics. There are two specific serotypes under O1 serogroup, Inaba and Ogawa, which are still surviving and a third unstable serotype under the O1 serogroup named Hikojima [11],[13]. Inaba has a terminal N-acetylglucosamine (GlcNAc) residue, while Ogawa has a terminal N-acetylgalactosamine (GalNAc) residue. Hikojima has both GlcNAc and GalNAc residues. Classical biotype was responsible for the first six pandemics of cholera from 1817 to 1923. El Tor biotype emerged in 1961 and caused the seventh pandemic of cholera that is still ongoing [10].

Vibrio cholerae is a MDRO [10],[11],[12]. Bacteria that have developed resistance to at least one bacteria in three or more antimicrobial categories are called Multidrug resistant organisms (MDROs) [6],[7]. MDROs pose a serious threat in the department of public healthcare. It can be present on the skin, in the ear or nose and other body parts and can spread between animals and humans [8]. Globally, cholera is thought to cause between 1.4 and 4.3 million infections and 28,000 and 142,000 fatalities per year [13]. Antibiotic resistance in V. cholerae is a major public

health concern, as it reduces the effectiveness of the available treatment options and increases the risk of complications and mortality. Cholera outbreaks have the potential to happen in countries where the disease is commonly found (endemic) as well as in countries where it is not prevalent (non-endemic), particularly in times of humanitarian emergencies, natural calamities, or conflicts. Preventing and managing cholera involves various measures such as ensuring access to clean water and sanitation facilities, promoting good hygiene practices, implementing surveillance systems for early detection, promptly responding to outbreaks, administering oral cholera vaccines, and providing treatment through oral rehydration solution and antibiotics for affected individuals. An unexpected rise in cholera cases was discovered in Bangladesh and Pakistan since the beginning of 2022 [18]. This led to the requirement for investigating the antibiotic resistance profile of *Vibrio cholerae* strains responsible for the small outbreak which took place during April 2022 in Bangladesh [18]. Investigation of the antibiotic resistance profile of *Vibrio cholerae* will allow doctors to have a better treatment plan for cholera patients. In this study we have investigated the serotype of the *Vibrio cholerae* strains and their antibiotic resistance profile.

2. Methodology

2.1. Collection of Bacterial Strains

Rice watery stool samples were collected from potential Cholera patients who came to the Dhaka Cholera Hospital of the ICDDR,B and sent to the Molecular Ecology and Metagenomics Laboratory of ICDDR,B in Dhaka during the year 2022. All the samples were screened for common enteric pathogens such as Enterotoxigenic Escherichia coli and V. cholerae. Before collecting stool samples, patients' or minor patients' legal guardians' informed consent was acquired. Among all the samples collected throughout the year 2022, 30 samples were randomly selected across 12 months of the given year. The stool samples were enriched in alkaline peptone water for 6 hours in 37℃. The enrichment samples were grown on Thiosulfate citrate bile salts sucrose (TCBS) agar plates and isolated to get pure culture by subculturing. The TCBS agar plates were streaked using Vibrio cholera isolates and incubated at 37℃ for 24 hours and a result is shown in figure 1. V. cholerae colonies were confirmed using a combination of biochemical, serological and molecular methods. Yellow colonies of *V. cholerae* are collected and further subcultured on Gelatinase agar plate (GA) plate. The samples were also grown in Taurocholate tellurite gelatin agar (TTGA) and incubated overnight. Black centered transparent colonies were selected for further subculturing on Gelatinase agar plate (GA) plate.

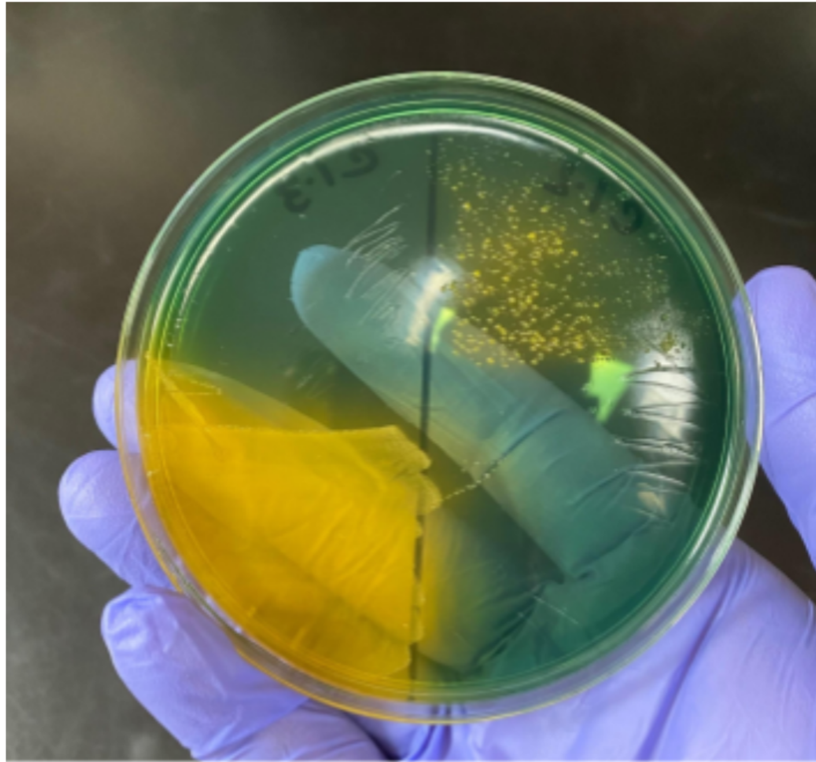


Figure 1. *Vibrio cholerae* on TCBS agar; Yellow colonies are *Vibrio cholerae* and Greenish colonies are other *Vibrio spp.*

2.2. Classification of Strains (Serotyping)

Serotyping was conducted by a slide agglutination test to confirm which serogroups the 30 *V. Cholerae* strains belonged to. For this *V. cholerae* O1 and O139 specific polyvalent antisera were used. Furthermore, the isolates showing a positive reaction to O1 specific polyvalent antisera were further tested to detect their serotypes using Inaba and Ogawa specific monoclonal antisera. The results differentiated their serogroups accordingly depending on respective agglutination formed [19],[20]. PCR was done targeting the O genes, O1- (rfbO1) and O139- (rfbO139), which further reconfirmed the serogroups.

2.3. Polymerase Chain Reaction (PCR)

As mentioned in the previous section, PCR analysis was performed to detect genes rfbO1 and rfbO139. The purpose was to confirm whether these strains belong to the O1 serotype [14],[19]. tcpA (encoding toxin co-regulated pilin A) was targeted to determine whether the strains

belonged to the El Tor or the Classical biotype [15],[19]. Separate conventional PCR was done. Agarose gel electrophoresis was done to observe the results of the PCR products.

The *Vibrio cholerae* O1-rfb specific primers used were O1F [5'- GTT TCA CTG AAC AGA TGG G -3'], sense strand and O1R [5'- GGT CAT CTG TAA GTA CAA C -3'], antisense strand. The *Vibrio cholerae* O139-rfb specific primers used were O139F [5'- AGC CTC TTT ATT ACG GGT GG -3'], sense strand and O139R [5'- GTC AAA CCC GAT CGT AAA GG -3'], antisense strand [14]. Just like the test conditions runned by Hoshino et al. (1998), our amplification condition used also was 5 min at 94°C for initial denaturation of DNA and 35 cycles, each consisting of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, with a final round of extension for 7 min at 72°C.

The primers used to detect the El tor biotype targeting the tcpA ET gene were ET-F [5'-CAC GAT AAG AAA ACC GGT CAA GAG-3'], sense strand and ET-R [5'-CGA AAG CAC CTT CTT TCA CAC GTT G-3'], antisense strand [15]. The primers used to detect the Classical biotype targeting the tcpA CL gene were CL-F [5'-CAC GAT AAG AAA ACC GGT CAA GAG-3'], sense strand and CL-R [5'-TTA CCA AAT GCA ACG CCG AAT G-3'], antisense strand [15]. We adopted the same test conditions as Rivera et al. (2001), where our denaturation was at 94°C for 2 min, annealing was set for 1 min at 60°C and extension at 72°C with a final extension step at 72°C for 10 min at the end of 30 cycles. *V. cholerae* O1 strains N16961 (ET biotype) and O395 (CL biotype) served as controls. The PCR primers used for the targeted genes are described in Table 1.

Table 1

Primers	**Sequences**	**Target Gene**	**Annealing temperature (℃)**	**Amplicon Size (bp)**	**Reference**
rfbO1 F	GTT TCA CTG AAC AGA TGG G	*rfbO1*	55	192	Hoshino et al. (1998)
rfbO1 R	GGT CAT CTG TAA GTA CAA C	*rfbO1*	55	192	Hoshino et al. (1998)
rfbO139 F	AGC CTC TTT ATT ACG GGT GG	*rfbO139*	55	449	Hoshino et al. (1998)

rfbO139 R	GTC AAA CCC GAT CGT AAA GG	*rfbO139*	55	449	Hoshino et al. (1998)
tcpA class F	CAC GAT AAG AAA ACC GGT CAA GAG	*tcpA* CL	60	620	Rivera et al. (2001)
tcpA class R	TTA CCA AAT GCA ACG CCG AAT G	*tcpA* CL	60	620	Rivera et al. (2001)
tcpA ET F	CAC GAT AAG AAA ACC GGT CAA GAG	*tcpA* ET	60	453	Rivera et al. (2001)
tcpA ET R	CGA AAG CAC CTT CTT TCA CAC GTT G	*tcpA* ET	60	453	Rivera et al. (2001)

2.4. Kirby-Bauer Disk Diffusion

Disk diffusion, as reported by the Clinical and Laboratory Standards Institute, was used to test antibiotic susceptibility using commercial antibiotic discs. Susceptibility to antimicrobials was determined on Muller-Hinton agar. The results were reported as S, I, R (sensitive, intermediate, and resistant) by a method based on the cutoff of the zone size for different antibiotics according to the latest available Clinical and Laboratory Standards Institute guidelines for V. cholerae [10],[11],[20]. 15 antibiotic discs were used. All strains of V. cholerae were tested for resistance to Aztreonam, Ampicillin, Chloramphenicol, Gentamicin, Ceftriaxone, Cefepime, Ciprofloxacin, Levofloxacin, Nalidixic acid, Imipenem, Azithromycin, Erythromycin, Trimethoprim sulfamethoxazole, Amoxicillin-clavulanic acid and Tetracycline using commercially available discs. A sterile cotton swab was used to lawn our isolated Vibrio cholerae strains on Mueller-Hinton agar plates. The inoculation plates were covered with antibiotic discs [11]. The plates were incubated at 37℃ for 18-24 hours. The antibiotic classes of the antibiotics used are shown in table 2.

Table 2

Names of Antibiotics	Code	Antibiotic class
Aztreonam	ATM	Monobactam
Ampicillin	AMP	Aminopenicillin
Chloramphenicol	C	Phenicols
Gentamicin	CN	Aminoglycosides
Ceftriaxone	CRO	3rd generation cephalosporin
Cefepime	FEP	4th generation cephalosporin
Ciprofloxacin	CIP	Fluoroquinolone
Levofloxacin	LEV	Fluoroquinolone
Nalidixic acid	NA	1st generation quinolones
Imipenem	IPM	Carbapenem
Azithromycin	AZM	Macrolides
Erythromycin	E	Macrolides
Trimethoprim sulfamethoxazole	SXT	Sulfonamides-Trimethoprim
Tetracycline	TE	Tetracycline
Amoxicillin-clavulanic acid	AMC	Penicillin

The zone widths of inhibition were measured in millimeter-scale (mm). Following the Clinical and Laboratory Standards Institute (CLSI) recommendations, samples were evaluated for antibiotic resistance. Table 3 includes the critical value or zone of inhibition values and concentration of antibiotic in the respective antibiotic discs used in this present study.

Table 3

Names of Antibiotics	Code	Concentration	Critical Value (mm)		
			Sensitive (S)	Intermediate (I)	Resistant (R)
Aztreonam	ATM	30	≥21	18 - 20	≤17
Ampicillin	AMP	10	≥17	14 - 16	≤13
Chloramphenicol	C	30	≥18	13 - 17	≤12

Gentamicin	CN	10	≥15	13 - 14	≤12
Ceftriaxone	CRO	30	≥23	20 - 22	≤19
Cefepime	FEP	30	≥25	N/A	≤18
Ciprofloxacin	CIP	5	≥21	16 - 20	≤15
Levofloxacin	LEV	5	≥17	14 - 16	≤13
Nalidixic acid	NA	30	≥19	14 - 18	≤13
Imipenem	IPM	10	≥23	20 - 22	≤19
Azithromycin	AZM	15	≥13	N/A	≤12
Erythromycin	E	15	≥17	13-16	≤12
Trimethoprim sulfamethoxazole	SXT	25	≥16	11-15	≤10
Tetracycline	TE	30	≥15	11-14	≤11

3. Results

All the strains used in this study produced typical *Vibrio cholerae* colonies on TCBS agar and biochemical reaction characteristics similar to that of *Vibrio cholerae.* Serological testing was conducted and it concluded that among the 30 strains studied, 46.67% (n=14) belonged to serotype Inaba and 73.33% (n=16) belonged to serotype Ogawa and all the strains belonged to O1 serogroup. The results of the serology test are included in table 4.

Table 4

Strain ID	**Polyvalent O1**	**Monovalent Inaba**	**Monovalent Ogawa**	**Serotype**
VC-1.3	+	-	+	Ogawa
VC-1.4	+	+	-	Inaba
VC-1.5	+	+	-	Inaba
VC-1.9	+	+	-	Inaba
VC-1.10	+	-	+	Ogawa
VC-2.6.1	+	+	-	Inaba
VC-2.6.2	+	-	+	Ogawa

VC-3.7.1	+	+	-	Inaba
VC-3.7.2	+	-	+	Ogawa
VC-3.13	+	-	+	Ogawa
VC-3.14	+	+	-	Inaba
VC-3.27	+	+	-	Inaba
VC-4.5	+	-	+	Ogawa
VC-4.19	+	+	-	Inaba
VC-4.24	+	+	-	Inaba
VC-5.10	+	+	-	Inaba
VC-5.16	+	+	-	Inaba
VC-5.17.1	+	-	+	Ogawa
VC-5.17.2	+	+	-	Inaba
VC-5.22	+	-	+	Ogawa
VC-5.29	+	+	-	Inaba
VC-6.8	+	-	+	Ogawa
VC-6.20.1	+	+	-	Inaba
VC-6.20.2	+	-	+	Ogawa
VC-7.28	+	-	+	Ogawa
VC-8.8	+	-	+	Ogawa
VC-8.21	+	-	+	Ogawa
VC-9.6	+	-	+	Ogawa
VC-9.19	+	-	+	Ogawa
VC-9.27	+	-	+	Ogawa

The reconfirmation of *Vibrio cholerae* O1 strains were done through PCR testing targeting their rfbO1 and rfbO130 genes and observed by gel electrophoresis. The *Vibrio cholerae* strains investigated in the present study are shown in table 5 including their date of collection and the

PCR results for the detection of serogroup and biotype. All 30 *Vibrio cholerae* isolates were *Vibrio cholerae* O1 strains which reconfirmed the serology results. The PCR targeted their tcpA ET and tcpA CL gene and 29 of them turned out positive for being El tor biotype and one of them (VC-9.27) had inconclusive results (which required further testing).

Table 5

Strain ID	Collection Date	rfbO1	rfbO139	Serogroup	tcpA ET	tcpA CL	Biotype
VC-1.3	3/1/2022	+	-	O1	+	-	El Tor
VC-1.4	4/1/2022	+	-	O1	+	-	El Tor
VC-1.5	5/1/2022	+	-	O1	+	-	El Tor
VC-1.9	9/1/2022	+	-	O1	+	-	El Tor
VC-1.10	10/1/2022	+	-	O1	+	-	El Tor
VC-2.6.1	6/2/2022	+	-	O1	+	-	El Tor
VC-2.6.2	6/2/2022	+	-	O1	+	-	El Tor
VC-3.7.1	7/3/2022	+	-	O1	+	-	El Tor
VC-3.7.2	7/3/2022	+	-	O1	+	-	El Tor
VC-3.13	13/3/2022	+	-	O1	+	-	El Tor
VC-3.14	14/3/2022	+	-	O1	+	-	El Tor
VC-3.27	27/3/2022	+	-	O1	+	-	El Tor
VC-4.5	5/4/2022	+	-	O1	+	-	El Tor
VC-4.19	19/4/2022	+	-	O1	+	-	El Tor
VC-4.24	24/4/2022	+	-	O1	+	-	El Tor
VC-5.10	10/5/2022	+	-	O1	+	-	El Tor
VC-5.16	16/5/2022	+	-	O1	+	-	El Tor
VC-5.17.1	17/5/2022	+	-	O1	+	-	El Tor
VC-5.17.2	17/5/2022	+	-	O1	+	-	El Tor
VC-5.22	22/5/2022	+	-	O1	+	-	El Tor

VC-5.29	29/5/2022	+	-	O1	+	-	El Tor
VC-6.8	8/6/2022	+	-	O1	+	-	El Tor
VC-6.20.1	20/6/2022	+	-	O1	+	-	El Tor
VC-6.20.2	20/6/2022	+	-	O1	+	-	El Tor
VC-7.28	28/7/2022	+	-	O1	+	-	El Tor
VC-8.8	8/8/2022	+	-	O1	+	-	El Tor
VC-8.21	21/8/2022	+	-	O1	+	-	El Tor
VC-9.6	6/9/2022	+	-	O1	+	-	El Tor
VC-9.19	19/9/2022	+	-	O1	+	-	El Tor
VC-9.27	27/9/2022	+	-	O1	-	-	Inconclusive (requires further testing)

The strains were resistant to the antibiotics IPM, SXT, AMP, AMC. An antibiotic resistance pattern had been observed with our results. 25 out of the 30 strains were found to be multidrug resistant (MDR). The antibiotic resistance pattern of the isolated *V. cholerae* strains (n=30) are shown in table 6.

Table 6

Resistance Pattern	**Number of strains**
IPM, SXT, AMP	19
IPM, SXT, AMP, AMC	4
STX, AMC, AMP	2
IPM, SXT	1
SXT, AMP	2
SXT	1
AMP	1

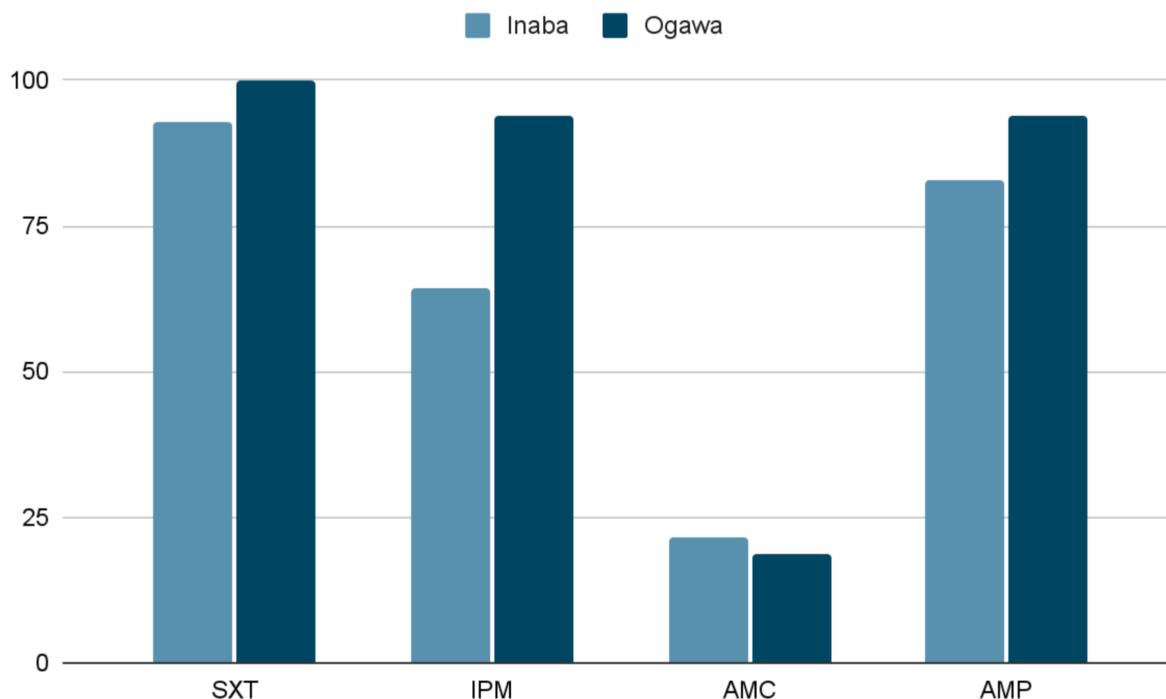
The most resistant antibiotics against our studied *Vibrio Cholerae* strains were SXT and AMP. Inaba serotype strains exhibited lower resistance percentages to IPM (64.29%) compared to Ogawa serotype strains (93.75%). No significant differences were observed in resistance to SXT, AMP, and AMC between the two serotypes. Table 7 shows their resistance percentages according to serotypes.

Table 7

Serotype	SXT (%)	IPM (%)	AMC (%)	AMP (%)
Inaba	92.86	64.29	21.43	92.86
Ogawa	100	93.75	18.75	93.75
Total *Vibrio Cholerae* (n=30)	96.67	80	20	93.33

A visual representation of the percentages of serotypes according to the antibiotics they are resistant to are shown in figure 2.

Figure 2



A two sample t-test was conducted to find significant differences between the 4 resistant antibiotics against the two serotypes. The table-8 shows the T-tests results.

Table 8

Antibiotic	t-value	p-value	Significant difference
IPM	-2.5364	0.0377	Yes
SXT	-1.3147	0.2093	No
AMP	-0.0894	0.9295	No
AMC	0.3529	0.7279	No

The "Significant Difference" column indicates whether there is a significant difference in resistance between the Inaba and Ogawa serotypes for each antibiotic. As per the t-test results, SXT, AMP, and AMC did not exhibit a significant difference. Statistical analysis using a two-sample t-test indicated a significant difference in IPM resistance between the Inaba and Ogawa serotypes of V. cholerae ($p < 0.05$).

4. Discussion

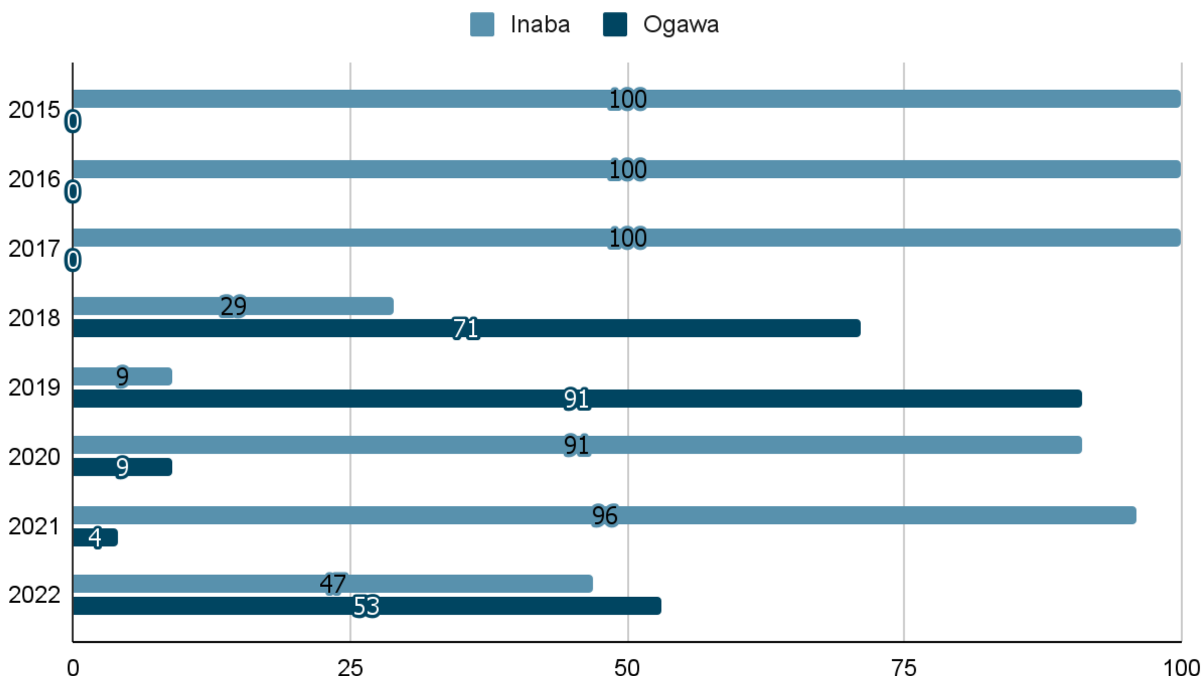
The isolates of *Vibrio cholerae* used in this study all resulted in being the biotype El tor after genotypic testing. V. cholerae O1 has two main biotypes: El Tor and classical. The classical biotype was responsible for the majority of cholera pandemics between 1817 and 1961; nevertheless, it eventually was replaced by the El Tor biotype, which first occurred in Indonesia in 1961 [28]. The El Tor biotype was found to be more resistant to phages and environmental stress than the classical biotype. Although the cholera toxin (CT) could be produced by both biotypes, they differed in the repressor genes that regulated the toxin's expression. In 1992, cholera epidemics were brought on by the emergence of the novel serogroup O139 from the El Tor biotype in Bangladesh and India [29],[30].

Multidrug resistant *Vibrio cholerae* isolates were found to have three different resistance patterns, which were, (i) IPM, SXT, AMP, (ii)IPM, SXT, AMP, AMC and (iii)SXT, AMC, AMP.

Imipenem, Trimethoprim sulfamethoxazole, Ampicillin and Amoxicillin-clavulanic acid belongs to the antibiotic classes Carbapenem, Sulfonamides-Trimethoprim, Aminopenicillin and Penicillin respectively. Organisms resistant to 3 or more different classes of drugs are termed as multidrug resistant (MDR). 83% of the isolates of *Vibrio cholerae* that underwent the antimicrobial confirmation tests were Multidrug resistant (MDR). Fluoroquinolone, Cephalosporin and Macrolides classes of antibiotics are used for treatment of Cholera [35] which were sensitive in our tests. Over the last few decades, *Vibrio cholerae* developed to be a notorious multi-drug resistant enteric pathogen [10],[11]. MDR *Vibrio cholerae* infections require prolonged treatment times and are challenging to treat. It is anticipated that the antibiotic resistance pattern will serve as a guide for choosing the best antibiotic for a given situation. In Bangladesh, antibiotics are sold with very limited oversight for which anyone can buy any antibiotic with or without a medical prescription [22]. Hence, in Bangladesh, due to uptake of unprescribed antibiotics, there is a high risk of development of MDROs. Both epidemics and pandemic cholera are caused by *Vibrio cholerae*. It poses a significant threat to low-economic groups with limited access to clean drinking water in many countries including Bangladesh.

The results of microbiological, biochemical and serological tests confirmed that all 30 *V. cholerae* isolates from cholera patients collected during the year 2022 were of O1 serogroup. Phenotypic tests confirmed that 47% of these strains belonged to Inaba serotype and 53% of these strains belonged to Ogawa serotype. Over the years, in Bangladesh, temporal changes have been observed on the serotypes of O1 *Vibrio cholerae* [10],[21]. Following on to the data provided by Jubayda et al. (2023), relating to the temporal changes between Inaba and Ogawa serotypes reported on isolates from the 2021 and isolates (from 2022) we investigated in the present study, we can see that in 2021, Inaba percentage was 96% and Ogawa percentage was 4% where as in 2022, Inaba percentage was 47% and Ogawa percentage was 53%. Figure 3 contains cross referencing of the percentages of Inaba and Ogawa strains between the years 2015-2021 provided by Jubayda et al. (2023) and the percentages of Inaba and Ogawa strains found in 2022 in this present study. The results of Jubayda et al. (2023) are compared with the results we obtained on the serotypes of *V. cholerae* which is shown in table 3.

Figure 3



Imipenem is an antibiotic classified under the carbapenem group, known for its broad-spectrum activity against various bacteria [33]. Nevertheless, certain strains of *Vibrio cholerae* may exhibit imipenem resistance, primarily attributed to the production of carbapenemase enzymes that can break down the antibiotic. [31]. Some *Vibrio spp.* showed significant resistance towards imipenem [32],[33]. Plasmid mediated enzymes are used to transmit antibiotic resistance genes to *V. cholerae* from other *Vibrio spp.* or fecal organisms like *E. coli* [12]. We have found a significant antibiotic (IPM) resistance difference between the serotypes of Vibrio cholerae O1 strains. Ogawa serotypes seem to be significantly more resistant towards IPM when compared with Inaba serotypes according to the results of t-test. The t- value and p-value of IPM resistant *V. cholerae* strains between its two serotypes are -2.5364 and 0.0377 respectively. P-value of IPM is less than 0.05 which indicates a significant difference. Further, genotypic investigation is required on this matter.

Following the restoration of the initial fluid deficit and prevention of vomiting, it is recommended to administer antibiotic treatment to cholera patients. In the earlier decades, specifically between the 1940s and 1960s, streptomycin and chloramphenicol were commonly and effectively used as antibiotics for the treatment of cholera [23],[24]. The efficacy of tetracycline in treating cholera

was demonstrated in Calcutta in 1962 [25]. Furazolidone was also considered as an alternative to tetracycline for the treatment of cholera in children, as it yielded comparable results in various clinical trials[26],[27]. In such a way, through clinical testing, different antibiotics were discovered to be useful against cholera over the years. But, Vibrio cholerae developed resistance against antibiotics for which different antibiotics are prescribed at different times by doctors to provide the best treatment. For doctors to provide the best treatment, they need to be aware of the antibiotic resistance patterns of Vibrio cholerae. According to Parvin et al. (2020), Vibrio cholerae, irrespective of serotypes, was most resistant to Tetracycline during the years between 2000-2004. After that, the resistance pattern of Vibrio cholerae towards tetracycline varied between the two serotypes. In the year 2021, Vibrio cholerae strains were not resistant to tetracycline [10] and again in 2022, it remains the same. A specific antibiotic, SXT, was resistant to Vibrio cholerae throughout the years between 2015-2021 [10] and it remained the same in 2022 based on the present study. AMP was not resistant to *Vibrio cholerae* in the years 2016, 2017, 2018 [10] and 2020. But, AMP was found to be resistant to *Vibrio cholerae* in the years 2019, 2021 [10] and 2022.

This research investigated the antibiotic resistance profile of *V. cholerae*. With proper antibiotic resistance pattern data available, doctors can prescribe the proper effective antibiotic treatment to the cholera patients.

5. Conclusion

In conclusion, our investigation supports the proportion of resistant isolates of *V. cholerae* collected for cholera patients. All the strains investigated were collected from different months of the year 2022 and all of them belonged to El tor Biotype of *V. cholerae* O1. This investigation reveals that most of the *Vibrio cholerae* strains collected within the year 2022 are resistant to Imipenem, Trimethoprim sulfamethoxazole, Ampicillin and Amoxicillin-clavulanic acid (which belonged to 4 different antibiotic classes) making them MDROs. Multidrug-resistant organisms pose a major threat with the treatment of infections. With proper antibiotic resistance investigations on MDR *V. cholerae,* we can provide a better insight in its antibiotic resistance pattern based on which healthcare experts can plan an accurate treatment plan for cholera.

6. References

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