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# Antimicrobial susceptibility of *Vibrio cholerae* Isolated from Diarrheal Patients in Najaf Province

**Zahraa Yosif Motaweq**
Faculty of Science, University of Kufa
***Corresponding author: E-Mail: zahraayosif@gmail.com**

**ABSTRACT**

Stool samples were gathered from patients with watery diarrhea matured (about 1 for >60 years) earlier organization to antibiotic operators who were alluded to Central Health Laboratory in Najaf. This study found that 27 isolate of *Vibrio cholerae* 52.3% male and 47.7% female. With respect to the serotyping of *V. cholerae* this research obtained whole isolates possess just Inaba serotype. The virulence factors were distinguished in all isolates was deliver β-hemolysis on blood agar. The highest one appeared at the (11-20 and 21-30) years age group with 29.6%, followed by others at age groups (>60) years and (41-50) years with 11.1%. The low isolation frequency was recorded at (51-60) years in a percent 3.7%. The consequences of this test demonstrated that *V. cholerae* has an extraordinary resistance to most commonly antibiotics utilized in hospitals, *V. cholerae* indicated diverse susceptibility towards antibiotics utilized in this study. The highest rate of resistance is seen with ampicillin 100%, nalidixic acid 89% and ciftazidime 85% and moderately resistance to pipemidic acid 70%, tobramycin 67%, aztreonam 63%, cefotaxine 48%, amikacin 41% and norflaxacin 41% whereas is relatively lower resistance toward azithromycin 37%, erythromycin 33%, ceftriaxone 33%, chloromphenicol 22%, tetracycline 11% and ciprofloxacin 7.5%. The consequences of this study showed that *V. cholerae* isolates were obtained to be a remarkable sensitive to Imipenem (100%) as well.

**KEY WORDS:** Cholera, *Vibrio cholerae*, Virulence Factors, Antibiotic susceptibility.

## 1. INTRODUCTION

Cholera is an acute intestinal infection brought on by the bacterium *V. cholerae*, which creates an enterotoxin (cholera poison) which was causing serious diarrhea which portrayed by plentiful, non-painful, aqueous feces, regularly joined through spewing that can rapidly prompt to extreme dryness additionally decease if therapy is not immediately presented. At the point when cholera happens in a ready society, mortality averages might be as high as half for the most part on the grounds that there are no easiness for therapy, or on the grounds that therapy is presented post factum. In spite of principally influencing developing countries, cholera remains a genuine general medical issue for some developed countries.

*V. cholerae* is a gram -ve bacterium curved rod, the initially portrayed by Pacini in 1854 however the initially disengaged by Koch in 1883, they were facultative anaerobic, very motile, nonspore framing bacteria. The majority essential serovar of serological was based on O1 which further sub partitioned into Inaba and Ogawa sero groups. It is for the most part acknowledged that eight particular pandemics of cholera have occurred ever after the starting of the primary pandemic in 1817 in Bangladesh.

As per the expected presentation, this review was wanted to achieve the accompanying points: 1- Isolation and distinguishing proof the pathogen from patients. 2- Recognition of antibiotic sensitivity profile for clinical isolates.

## 2. MATERIALS AND METHODS

**Patients and Clinical Specimens:** A sum of 153 stool specimens were gathered from patients experiencing watery diarrhea earlier organization to antimicrobial agents who were alluded to Al-Sadder Medical City, Al-Hakeem General Hospital and Al-Zahra Hospital for Childbirth and Children in Al-Najaf governorate through the interval from August to November 2015. The patients comprised sex (male and female), location and the age range (1-80 years).

All the samples were collected into Cary-Blair transport medium and delivered to the Regional Hospitals where further investigations in form of standard microbiological methods utilized for the culture and recognizable of *V. cholerae* isolates. Samples were inoculated in alkaline peptone water broth APW at 37°C for 6 to 8 h before plating. 5 µl from the upper layer of APW was taken and then streaked onto MacConkey agar, TCBS agar and blood agar then incubated for 18 to 24 h at 37°C. Two-three colonies with the attributes semblance (2 to 3 mm in diagonal, yellow, and level) were subjected for further identification. Any samples with negative culture would be precluded from the research.

**Isolation and Identification of *V. cholerae*:** *V. cholerae* was isolated and identified according to traditional biochemical diagnostic to, by using the routine methods e.g. according to Macfaddin.

Biochemical identification done according to:

**Oxidase test:** The experiment was achieved according to. Oxidase-positive strains were further identified with commercially available serological tests and with the API 20 E system.

**String test:** was used to differentiate *Vibrio* species (spp.) from *Aeromonas* spp and *Plesiomonas shigelloides* and done especially for cultures that similar to *V. cholerae* but dud to agglutinate in demonstrative antisera. The string test done according to.

**API-20 E:** Likewise the isolates affirmed biochemically by utilizing API 20-E system (Biomereumix/France) as per guidelines of provided organization.

- **Biotyping:** The biotype of *V. cholerae* serogroup O1 strains was recognized by the accompanying procedures; polymyxin B sensitivity, hemolysin test, and Voges-Proskauer test.
- **Serotyping:** Serologic affirmation was carried out by utilizing polyvalent, anti-Ogawa, anti-Inaba antisera (Plasmatec Laboratory Products Ltd) (Plasmatec/UK) and O139 antisera (GlaxoWellcome, England). Serotyping according to Baumman and Schubert, 1984; Elliot, 2001 and WHO (2002). Cultures that look like *V. cholerae* but neglect to agglutinate in demonstrative antisera (non agglutinable or non-O group 1 vibrios) will be subjected to additional tests such as decarboxylases and the "string test.

**Virulent factor detection:** Haemagglutination Test (HA): Red blood cell suspension was set up from the group A human RBC. RBC laundered with phosphate buffer saline (frequented 3 times), 3% suspension from Red blood cell (V/V) was then readied and concurrently, blood agar was readied, then it was inoculated with *V. cholerae* and incubated at 37C˚ for 24 hrs, subsequently, place 1 drop of human RBCs with loopful onto an immaculate slide, to this, drop additive 1 drop of *V. cholerae* culture by utilizing a blazed loop were blended with human RBC on immaculate slide. The blood agglutination with *V. cholerae* was distinguished in 25C˚ through (1 to 5) min. Agglutinated RBCs in suspension (+ve reaction) had a clustered emergence particular from non-agglutinated RBCs (-ve reaction).

- Detection of Toxin coregulated pili (TCP): *V. cholerae* hydrophobicity augmented in broth culture because of the manifestation of pili bringing about noticeable clustering of bacteria as a pellet at the base of tube and leaving a limpid supernatant; this event is defined auto agglutination which detected by macroscopical examination.
- Production of Protease: *V. cholerae* were streaked on TSA containing either 1% casein or 1% tween 80 or 7% sheep blood and incubated for 18-48 hour at 37°C. Cleared zone was used to detect proteases.

**Antibiotic sensitivity test:** Disk agar dissemination in accordance with the Kirby–Bauer standardized antimicrobial sensitivity single disk method was accomplished.

## 3. RESULTS AND DISCUSSION

**Isolation and Diagnosis of *V. cholerae:*** Twenty seven *V. cholerae* isolates were obtained from patients suffering from cholera disease from the Central Health Laboratory in Najaf. Identification of *V. cholerae* were first made by the bacteriological methods including colonial morphology, Gram stain and other biochemical tests. In culture, identification of *V. cholerae* depended on the colonial morphology and the presence of hemolysis type β on blood agar plate, since *V. cholerae* form large yellow colonies on TCBS agar readily visible against the dark-green background of the agar. This medium was alkaline pH (8.6) supports growth of *Vibrio spp*., particularly that of *V. cholerae*. Oxgall and sodium cholate were comprised to repress the growth of Gram-positive bacteria. Sucrose was the fermentable carbohydrate and sodium thiosulfate was comprised as an electron acceptor for sulfur reducers. Bromthymol blue was the pH pointer and ferric ammonium citrate was comprised to demonstrate sulfur shorthand. TCBS Agar was a selective and differential medium utilized for the initial dissociation of *Vibrio* species. *V. cholerae* was sucrose fermenters producing acid end-products form yellow colonies in this medium. *V. cholerae* was growing on MacConkey agar and colonies appeared colorless or pale, medium size when incubated for 24 hrs. But when accessed incubation time to 48 hrs or more, observed colonies color shift to the pink color of low degree. This evidence was late lactose fermenter.

In microscopically examination by (Gram stain) of colony from culture, the typical *V. cholerae* appeared in straight or curved rod Gram-negative, arranged in pairs or in short chains. The consequences of biochemical tests that shown in table (1) were regarded as a supplementary of the primary definition of *V. cholerae* isolates. The isolates fit to general properties that were oxidase negative and catalase positive. They were motile, indole, methyl red and urease positive. The isolates were capable to ferment glucose on KIA agar, it produced (Alkaline) red color on slant and bottom (acidic) yellow color with not produce gas or $H_2S$ precipitate.

**Table.1. Biochemical test of *V. cholerae* suspected isolates**

| Test | Standard result |
|---|---|
| Oxidase test | + |
| Catalase test | + |
| Cholera red test | + |
| Indol production test | + |
| Methyl red test | + |

| | |
|---|---|
| Urease production test | - |
| String test | + |
| Blood hemolysis test | β-hemolysis |
| KIA agar growth | Alk/A<br>$H_2S^{-ve}$<br>$Gas^{-ve}$ |

In string test that considers important tests depended in *Vibrio* diagnosis where most isolates shows positive results for this test. A positive reaction was shown by the suspension during 1minute: it absences its turbidity and gets to be distinctly mucoid, a "mucoid string" can be towed if the consequence was positive, the bacterial cells will be decomposed by the sodium deoxycholate, the suspension will lack turbidity, and DNA will be liberated from the lysed cells giving rise to the blend to turn into distinctly gooey. A mucoid "string" was shaped when an inoculating loop is towed tardily far from the suspension when the loop was tardily elevated far from the drop.

API 20E was affirmation utilized to diagnose all clinical isolates. This was a highly accurately in diagnosis, a rapid, reliable and simple test for reaching the species.

**Serotyping diagnosis of *V. cholerae*:** In trying to knowing the ascendancy of particular serotype of *V. cholerae* between the clinical isolates encompassed in the current research, consequences indicated plainly that Inaba serotype had prevailed in all clinical isolates of *V. cholerae* with a rate 100%.

In 2006, 2007 additionally 2008 detected that Inaba serotype is the prevailing of serotype of *V. cholerae* in Iraq and respectively. This consequence was in convention with a research in Pakistan by. It have been registered that, there has been a huge increment in Inaba serotype, in spite of the fact that the dominating *V. cholerae* serotype in the earlier years (1993-2005) is Ogawa and serotype Inaba is uncommon. Hasson (2012), recorded that all isolates have Inaba serotype.

**Prevalence of *V. cholerae* according to risk factors:** In the current study, the prevalence of *V. cholerae* isolates based on patient's age in different groups was shown in figure.1. The highest one appeared at the (11-20 and 21-30) years age group with 29.6%, followed by others at age groups (>60) years and (41-50) years with 11.1%. The low isolation frequency was recorded at (51-60) years in a percent 3.7%.

Hasson (2012), indicated that all ages were influenced; however the preponderance of patient ages were among > 1 and 20 years. These discoveries are identical to those detailed from other cholera-endemic region, where the most noteworthy occurrence of clinical cholera is generally seen between little children, pre-school kids and ladies of childbearing age in Iraqi study that is probably going to be attached to the decreased level of immune efficiency at this stage.

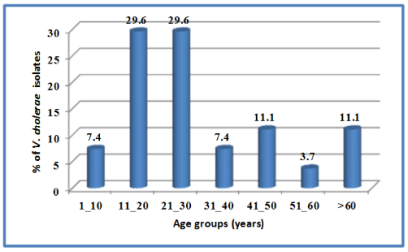


**Figure.1. Prevalence of *V. cholerae* according to the age**

According to sex, there was no critical variation (P>0.05) among cholera isolates and type of sex. However *V. cholerae* isolated from males in a percentage of (55.5%) which was more than females (44.5%) according to displayed in Figure.2.

This result is like to Iranian research which discovered that questionable patients with cholera were 58% males and 42% females additionally to Al-Abbassi (2005), research that discovered there is no distinction among infected male and female.

This study is agreement with Hasson (2012), discovered that 44 dubious isolates of *V. cholerae* 52.3% male while 47.7% female.

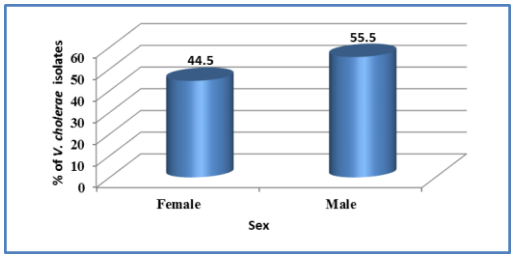


**Figure.2. Prevalence of *V. cholerae* according to the sex**

There was significant variation (P>0.05) between residence and cholera infection (figure.3).

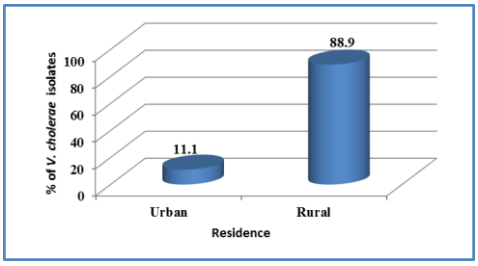


**Figure.3. Prevalence of *V. cholerae* according to the residence**

**Virulence Factors of *V. cholerae:*** It is recognized that the pathogenicity of *V. cholerae* is related to many virulence factors. In this study, some of them were detected by traditional phenotypic methods.

**Detection of β-Hemolysine:** All *V. cholerae* isolates (100%) in this study presented β-hemolysis appearance on human blood agar. The appearance of a hemolysis region (β-hemolysis) around colonies shows that the colonies have the ability to hemolyze red blood cells. β-hemolysis, the total annihilation of RBCs and hemoglobin, consequences in a clearing of the medium about the colonies. Numerous bacteria liberation substances that reason hemolysis of RBCs, and this specialty have been supposed to be an serious virulence characteristic. Actually, some bacterial hemolysins were illustrative of common classes of bacterial exotoxins (the cytotoxins) that murder host cells by damage the host cell membrane. Additionally, hemolysins may release important growth factors, for example, iron for the attacking microorganisms.

There are two biotypes (subdivisions) of the species *V. cholerae*: classic and El Tor. Rather than the classic strain, the El Tor strain was featured by the creating of hemolysins, greater transmission proportions, and the capacity to stay alive in water for protracted intervals. The El Tor hemolysin was oligomers, however not that of the monomers, to the watery stage swimming lipid bilayer membranes reached in the construction of ion- porous ducts, which had long lifetimes at low voltages. The hemolysin ducts had a solitary-duct disposal of 350 pS in 1 M KC1. These outcomes characterized hemolysin (HlyA) from *V. cholerae* as a duct-constituting ingredient with characteristics like to other cytolytic poisons. The monocular-duct conductance of the hemolysin (HlyA) from *V. cholerae* 01 biotype El Tor duct was a straight assignment of the mass watery conductance, which proposed that the poison composes watery ducts with an expected least distance across of around 0.7 nm. The hemolysin duct of *V. cholerae* was observed to be sparingly anion-eclectic. All these cytolytic poisons should most likely oligomerize for action in biological and synthetic membranes and compose anion- eclectic ducts.

**Detection of TCP:** The results of the current study gives that all isolates (100%) possess TCP, which is evident on the grounds that TCP functions as a CTX phage receptor on one hand and as an adherence agent on the other hand. *V. cholerae* hydrophobicity excessed in broth culture because of the expression of pili causing apparent clustering of bacteria as a pellet at the base of tube and remainders a transparent supernatant this marvel is recognized as auto agglutination.

The result of present study is concordant with Saleh (2011), who pointed out that 100% of isolates showed TCP activity.

**Detection of Protease:** In the present study, show 85% (23/27) of clinical isolates produce protease. The consequences of research are in concurrence with Qu (2003) and Saleh (2011), which recorded that 80% and 90% of clinical isolates showed protease activity respectively. *V. cholerae* create Zn-subordinate metaloprotease (mucinase); which is additionally dubbed haemaglutinine/HA protease that is included in the proteolytic invigoration of poisons, for example, CT, cytolysin, El-Tor haemolysin and hydrolysis of numerous major proteins like mucin, lactoferrin, fibrinectin. Proteases additionally perform a necessary function in mucous obstruction permeation to arrive bacteria to superficies of epithelial cells that coating short intestine and reason disease.

The consequences of present research came in harmonization with Bag (2008), who detected that 80% of clinical isolates showed protease activity. The essential of these enzymes in the relation of these enzymes with enterotoxin released which by all means is the poison in charge of creating cholera in human.

**Antimicrobial Susceptibility test:** Table.2, shows the phenotypic susceptibility of 37 *V. cholerae* isolates to (16) commonly used antimicrobial agents by using Kirby-Bauer disk diffusion method. The outcomes were translated by measurement of inhibition zone and compared with stander zones of inhibition determined by Patel (2014).

The consequences of this test demonstrated that *V. cholerae* has major impedance to usually antibiotics utilized in hospitals, *V. cholerae* demonstrated various sensitivity trend antibiotics utilized as a part of this study as appeared in table.2. The highest rate of resistance is appeared with ampicillin 27/27 (100%), nalidixic acid 24/27 (89%) and ciftazidime 23/27 (85%) and moderately resistance to pipemidic acid 19/27 (70%), tobramycin 18/27 (67%), aztreonam 17/27 (63%), cefotaxine 13/27 (48%), amikacin 11/27 (41%) and norflaxacin 11/27 (41%) whereas is relatively lower resistance toward azithromycin 10/27 (37%), erythromycin 9/27 (33%), ceftriaxone 9/27 (33%), chloromphenicol 6/27 (22%), tetracycline 3/27 (11%) and ciprofloxacin 2/27 (7.5%). Improvement of antibiotic

resistance is frequently attached to the excessive use, and the wrong use of the antibiotics specified. Resistance about *V. cholerae* keeps on being a necessary clinical curative trouble, like that of which could make discovered for an expanding multidrug resistance on these bacteria. The outcome of this study showed that *V. cholerae* isolates were detected to be a remarkable sensitive to Imipenem (100%) as well.

The current outcomes concurred with Al-Obidi who established that the *V. cholerae* isolates demonstrated a large susceptibility to every AMP, CIP, E, TE, CN Also KF, furthermore demonstrated a least resistance (10%) also (20%) should CTX furthermore Ak sequentially. It may be intriguing to discover that the consequences of the current research were near to the outcomes of other projects illustrated by some investigators, that every isolates of *V. cholerae* appeared an elevation susceptibility to AMP, TE, CIP and Samal (2008), showed pointed out that 144 *V. cholerae* isolates have 94.1% resistant to Na and 80.4% to FX . The consequences of current research was in agreement with the outcomes of Samal (2008), who demonstrated that *V. cholerae* isolates sensitive to C, CIP and TE.

**Table.2. Antibiogram of 27 *V. cholerae* isolates**

| Antibiotics | Symbol | S | | I | | R | |
|---|---|---|---|---|---|---|---|
| | | No. | (%) | No. | (%) | No. | (%) |
| Ampicillin/Cloxacillin | Apx | 0 | 0 | 0 | 0 | 27 | 100 |
| Amikacin | Ak | 3 | 11 | 13 | 48 | 11 | 41 |
| Azithromycin | AZM | 17 | 63 | 0 | 0 | 10 | 37 |
| Aztreonam | AT | 7 | 26 | 3 | 11 | 17 | 63 |
| Ceftriaxone | CTR | 13 | 48 | 5 | 19 | 9 | 33 |
| Cefotaxime | CTX | 14 | 52 | 0 | 0 | 13 | 48 |
| Ciftazidime | CAZ | 4 | 15 | 0 | 0 | 23 | 85 |
| Ciprofloxacin | CIP | 22 | 81 | 3 | 11 | 2 | 8 |
| Chloramphenicol | C | 17 | 63 | 3 | 11 | 6 | 22 |
| Erythromycin | E | 9 | 33 | 9 | 33 | 9 | 33 |
| Imipenem | IPM | 27 | 100 | 0 | 0 | 0 | 0 |
| Nalidixic acid | Na | 3 | 11 | 0 | 0 | 24 | 89 |
| Norfloxacin | Nor | 9 | 33 | 7 | 26 | 11 | 41 |
| Tetracycline | TE | 19 | 70 | 5 | 19 | 3 | 11 |
| Tobramycin | TOB | 0 | 0 | 9 | 33 | 18 | 67 |
| Pipemidic acid | PI | 8 | 30 | 0 | 0 | 19 | 70 |

## 4. CONCLUSION

The next conclusions are taken from the current study: 1- Clinical isolates of *V. cholerae* were serotyping as Inaba serotype. 2-The virulence factors were distinguished in all isolates was delivering β-hemolysis on blood agar. 3-The consequences of this test demonstrated that *V. cholerae* has an extraordinary resistance to most commonly antibiotics utilized in hospitals, *V. cholerae* indicated diverse susceptibility towards antibiotics utilized in this study. The highest rate of resistance is seen with ampicillin, nalidixic acid and ciftazidime. The consequences of this study showed that *V. cholerae* isolates were obtained to be a remarkable sensitive to Imipenem as well.

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