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EPIDEMIOLOGICAL STUDY AND ANTIBIOTIC RESISTANCE OF *VIBRIO CHOLERAE*

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ABSTRACT:

The study was conducted in Karbala city in Iraq,ncluding isolation and identification of *Vibrio cholerae* from diarrhea cases. Samples were collected from different hospitals in the city. The samples were cultured directly on Alkaline Peptone Water (APW), TCBS agar, and Blood agar, then the Vibrio was identified by microscopic and biochemical tests, and the biochemical panel from bioMerieux (APl20E) was used to confirm identification. Firstly, the serotype was identified by antisera and biochemical tests used to differentiate the biotype EI TOR and Classical. Spring test was used to differentiate Vibrio spp. from Aeromonas spp. Secondly, the statistical study of Cholerae epidemiology in Karbala province during 2015 to identify patient numbers and the most common serotypes. At the last step, determine the antibiotic susceptibility profile of *V. cholera* isolates against 12 antibiotics (Clindamycine, tetracycline, Pipracilline, Cefoxitin, amikacin, ampicillin, amoxicillin Clavanic acid, Nitrofurantoin, ticarcillin-clavulanic acid, erythromycin, Nalidic acid, and Cefapim). The phenotypic characteristics, biochemical test, APl20E, and VITEK SYS revealed that only 157 isolates out of 200 diarrhea samples was *V. cholerae* O1 EI TOR of INABA serotype. The epidemiological survey uncovered that 2015 was the higher year recording cholerae infection at 157 infections than other years, while no cholerae infection was recorded during the next years. INABA serotype was the most common serotype, and the diseases were recorded in males more than females at statistical significance ($p< 0.05$). The results showed that Karbala ranked 13 out of 17 epidemic provinces during 2015 compared to other cities, where The antibiotic susceptibility profile showed that all *V. cholerae* isolates were 100% sensitive to Amikacin, Nalidic acid, cifampim, and Nitrofurantoin while 100% resistant to Amoxicillin, Erythromycin, ticarcillin-clavulanic acid, Cefoxitin, ampicillin, clindamycin, and Tetracyclin while were moderate to pipracilline.

Keywords: *- Vibrio cholera,* Antibiotic resistance, Karbala province

INTRODUCTION

Cholera is caused by *Vibrio cholerae*, a bacteria that causes a severe diarrheal sickness. It is spread through polluted water and food and is seen worldwide [1]. Many people have died as a result of this disease during major epidemics. New cases and deaths of this disease are reported in various parts of the world, particularly in Africa and Asia. According to a World Health Organization study from 1995, cholera cases totaled 20, 8755, with 5034 deaths [2]. The most important reasons for the emergence of different dangerous waterborne diseases, including cholera, are undoubtedly contaminated water resources with sewage and wastewater due to a lack of effective collection and purification of water and a shortage of clean drinking water [3]. It's worth noting that in 1844, John Snow was the first to identify the link between contaminated water and

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cholera in England. As a result, polluted water is one of the most critical variables in cholera transmission, and in the case of contaminated drinking water, a huge number of individuals will become sick in a short amount of time, turning the disease into an acute epidemic. According to surveys of epidemics that have occurred around the world to date, the majority of these diseases are caused by sewage contamination of drinking water resources [4]. In other words, epidemics are more likely to occur in areas with poor water quality and sanitation [5]. Because water plays a role in the spread of this disease, the most important strategies to prevent the disease are to protect water resources from contamination, dispose of sewage and wastewater properly, provide clean drinking water, and monitor the health of the water supply [6].

MATERIAL AND METHOD

Patient:-

A. Samples Collection:

200 samples were collected from different age patients suffering from watery diarrhea, from both sexes in hospitals in Karbala provinceduring 2015. Fecal samples were placed in a sterile tube with Cary & Blair medium.

B. Data analysis

Data were collected from different sources Karbala health directories for V. Cholera epidemic episodes that occurred in years 2015

C. Identification of Isolates

The recognition tests involving cultural, morphological, and biochemical character (API 20 E and Vitek system) were done for every isolate. The test was carried out on the glass microscope slide by suspending TSA growth of 18-24 hours in a drop of 0.5 percent of aqueous Na-deoxyxy solution

When an inoculating loop is drawn slowly upwards from the suspension, which implies a positive outcome, the cholate, a mucoid string, is produced.

D. Serological Test:

V. cholera somatic antigens slide agglutination test was performed: using a saline fresh-growth suspension taken from non-selective media directly as TSA and distributed on the glass slide using a sterile application tool. The suspension then earned a slight reduction of polyvalent Ab (O1) (ca. 10 ml) and was well combined. Then you turn the slide back and forth to observe agglutination. In the case of positive samples, a strong agglutination happens within 30 sec to 1 min. Tests were also conducted with monovalent antiserum, Ogawa, and Inaba to establish the serotype.

E. Antimicrobial Susceptibility Test.

In this study, the choice of antibiotics and their standard inhibition diameters was used as suggested according to (Todar, 2005) [7] via the Kirby Bauer standard technique.Figure 1 shows the antimicrobial disks that were used to determine the susceptibility of *V.cholera* isolates to 12 different antibiotics (Manufactured by Oxoid England company)

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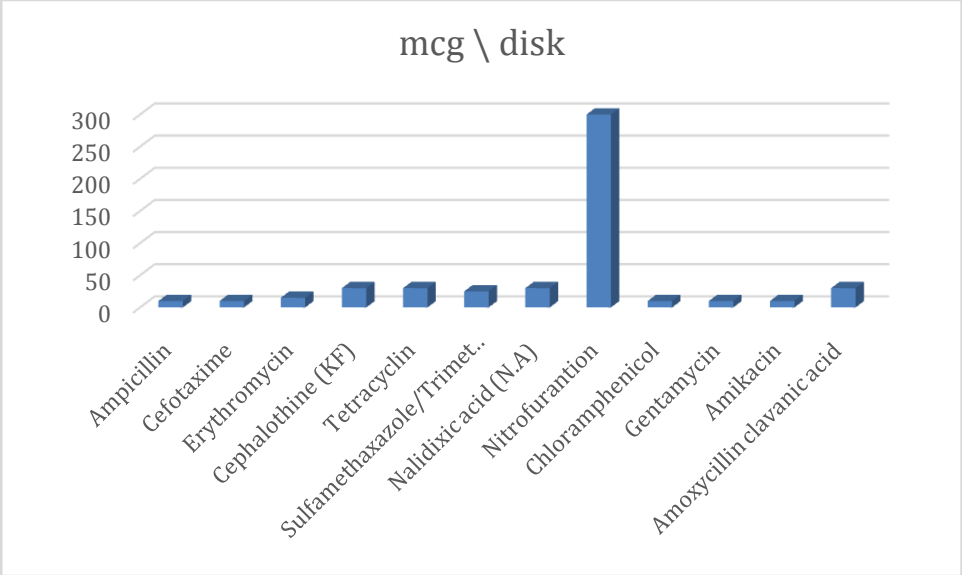

Figure (1): Antimicrobial disks.

Results

Based on data collection, *V. cholera* was an epidemic episode that occurred during 2015 in Karbala and other Iraqi cities, as shown in figure 3. According to the laboratory findings of this study, 157 (2.318 percent) of 200 diarrheic patients tested positive for *V. cholerae* bacterial culture. All of the isolates were found to be *V. cholerae* O1 ElTor, and they were identified using colony morphology, growth characteristics, biotyping methods, and serological tests, as well as a comparison of biochemical properties. Serotyping by using polyvalent cholera O1 antisera and monovalent Ogawa and Inaba revealed that most of clinical *V. cholerae* isolated from the stool of diarrheic patients of Karbala province were of serogroup O1, and all the agglutinating strains in this study were identified as *V. cholerae* O1, biotype El Tor, serotype Inaba.

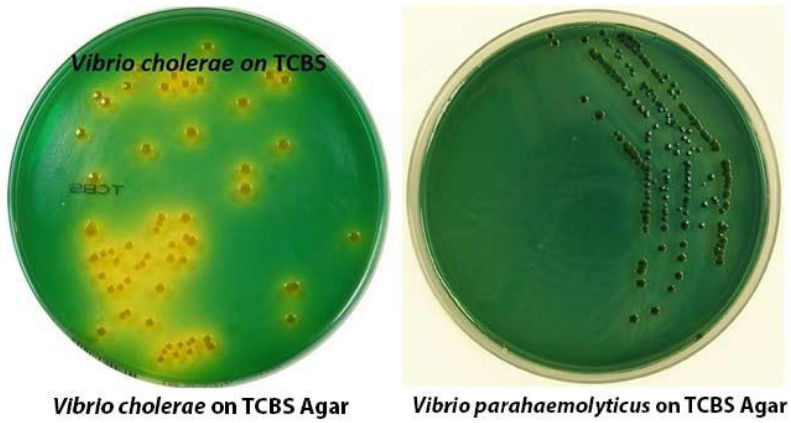

Figure (2)colony morphology of *V. cholerae* on TCBS agar

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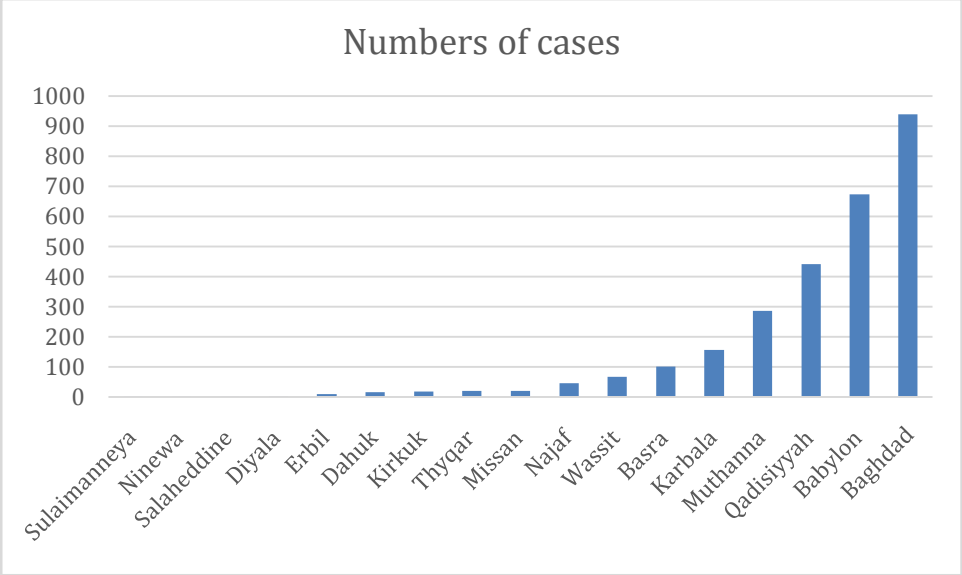

Figure (3) show the number of*V. cholerae*cases indifferent Iraqi cities during 2015

Biochemical identification:

To confirm the diagnosis of *V. cholerae* with the biochemical tests were used, *V. cholerae* was positive for catalase and oxidase, produced acid on TSI also positive for indole and negative for uereas test, while was positive for MR-VP and citrate utilization as results that are shown in figure 4 (API 20E system).

Figure (4) API20E System for *V. cholerae* isolates.

Vitek system identification:-

Among the biochemical tests were used for the identification of bacteria, vitek system for identification of V. *cholerae*. The analytical profile index of this system shows a probability of 99% identification percentage.Prevalence of*V. cholerae* according to gender.

The results show that the prevalence of *V. cholera* was higher incidence among males with percentage (65%) than females (35%), as shown in figure5. Based on F-test, the cases between gender distribution positive for *V. cholerae*are significant (P<0.05).

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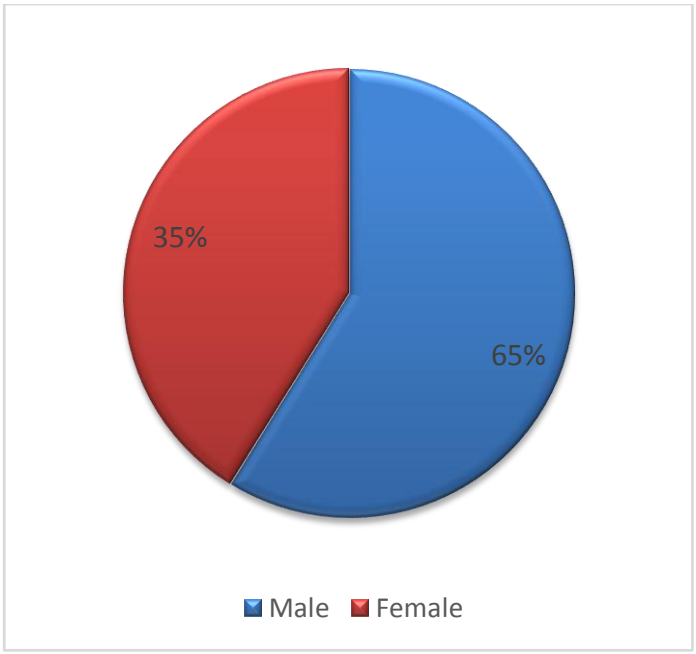
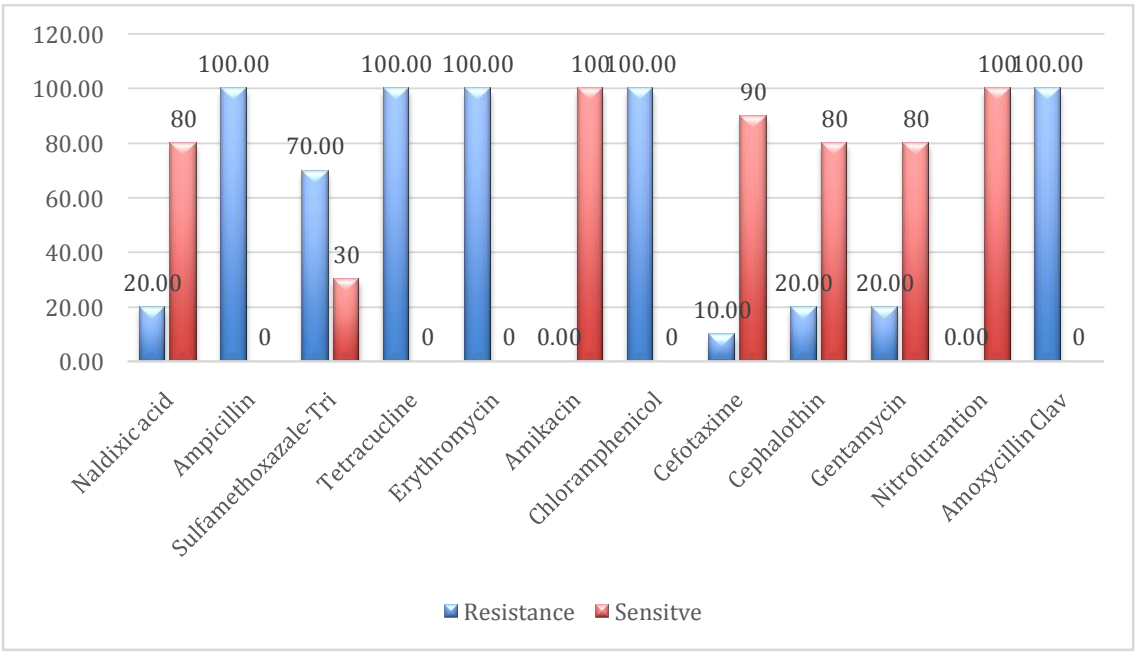


*significant (P<0.05)

Figure (5) show the prevalence of cholera in Karbala province according to gender.

Antibiotic sensitivity test:-

Figure (6) shows that all *V. cholerae* isolates are sensitive (100%) to Amikacin and Nitrofurane, whereas other *V. cholerae* isolates have resistance rates of 20% to Cephalothin, Gentamicin, and Nalidixic acid. *V. cholerae* isolates are also resistant (10%) to Cefotaxime and (70%) to trimethoprine-sulfa. Tetracycline, ampicillin, chloramphenicol, amoxicillin-clavanic acid, and erythromycin resistance are present in all *V. cholerae* isolates (100 %).



*significant (P<0.05)

Figure (6) resistant and sensitive of antibiotics discs against *V. cholerae* isolates

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Statistical F-test shows the differences in sensitivity for isolates are significant at level ($P<0.05$)

Discussion

Studies show that cholera infection is one of the triggers of diarrhea in men above one year of age. Stool culture. Bacterial detection. It can be seen that *Vibrio cholera*, which is an etiological agent in diarrhea and is specifically linked to poverty and bad sanitation, is contaminated by humans and pathogens. The conventional detection of *V. cholerae* is typically performed through a set of biochemical tests on a select plate medium (TCBS agar, for example) following their development and isolation [12]. Several studies in Iraq indicate that *Vibrio cholerae* prevalence was (42.9 %), (47.14%), (87 %) recorded by the Al-Obidi method and by the Kadhim system, respectively by AL-Karkhy by API 20E system; in reality, the Vibrionaceae family is considered appropriate for identification. While the capacity of commercial systems to classify the members of the Vibrio genus is concerned, relatively few records remain. The sequence of biochemical tests widely used in recognizing *V. cholerae*was initially developed for clinical samples [8]. (showed that direct use of the PCR method to classify a screened colony yielded stronger results than API20E method) [9].The incidence of *V cholera* in 2015 is greater than in others years. Results have demonstrated that the V-serotype is controlled by Inaba. The *V-cholerae* isolates from prior epidemic events and Globally diagnosed as *V. cholerae*Inaba. Previous studies between 2015 and 2017 V-serotype isolates may be proposed to have a general V-chaolerae endemisity. 2006, 2007, and 2008, respectively ,found that Inaba serotype is the dominant serotype of *V. cholerae*. From results show that the prevalence of *V. cholera* was higher incidence among males as compared with females. This might be due to the fact that most males are out – doored and from this point of view, they could be regarded as food eating and handling or contact with other patients as mentioned by Kadhim [10]. Possibly because rural patients tend not to be admitted to central hospitals for many reasons, such as (Financial and far distance from their residence) and because they may be treated in a local hospital in their districts as outpatients.

On 2 December of 2015, World Health Organization(WHO) reported that the pilgrimage of Arbaeen is going to take place in Karbala. A total of 10 million pilgrims are expected to attend. The National IHR Focal Point of Bahrain, the Islamic Republic of Iran, Jordan, Kuwait, Oman, Qatar, and the United Arab Emirates have put in place preparedness measures for the early detection and management of any imported cholera case from Iraq. Based on that the epidemic of *V. cholera* will be limited.

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