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Cholera distribution in different parts of Iraq during 2015 epidemic

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ABSTRACT— Cholera is an important, recurrent source of morbidity and mortality in many developing countries. Illness is caused by infection with toxigenic Vibrio cholerae O1 or O139 bacteria, most often acquired through ingestion of fecally contaminated water or food. Symptoms include nausea, vomiting, and profuse watery diarrhea. Severe disease causes rapid dehydration, is marked by loss of skin turgor and sunken eyes, and can result in death within hours if untreated. The first aim of this study was to review the prevalence of cholera infection in different governorates of Iraq during the period from 1/1 to 9/12 / 2015. Secondly, to clarify the governorates with the highest cholera incidence, and try to explain the factors behind this incidence if found. In this prospective cohort study, this was comprised of 2866 subjects out of 3547 examined cases. They were sent from different parts of Iraq, who were diagnosed with cholera infection. These cholera patients were collected during the period from January to December 2015. Bacteriology, serology and all other lab investigations were worked out in the central health laboratory in Baghdad. There were high cholera casualties' proportions in a number of Iraq governorates as Baghdad Al-Rusfa (n=627, 21.9 %%), Baghdad Al-Karkh (n=357, 12.5%), Al-Hilla (n=657, 23.6%), and Al-Diwanyia (n=445, 15.5%) compared to other parts of Iraq in the same period, and the disease seems to localize in middle and to lesser degree in southern parts of Iraq. We recommend repeating the study, in a larger frame, using more sophisticated tools, especially molecular diagnostics which have proven their value as extremely sensitive and specific techniques, that can improve the diagnosis of cholera and also help in putting a more accurate epidemiological characters of this disease.

KEYWORDS: Cholera

1. INTRODUCTION

Cholera is an acute form of diarrheal disease that afflicted human civilization over the centuries. The sudden and explosive onset of the disease in the form of an outbreak or epidemic, coupled with high mortality and morbidity rates, had a tragic impact on the personal as well as social life of people living in the affected areas [1]. The enormity of human sufferings led clinicians and scientists to carry out extensive research on cholera and *Vibrio cholerae* leading to major discoveries that opened up novel areas of research or new disciplines in biomedical sciences.

Vibrio cholerae is a Gram negative bacterium curved rod, it was first described by Pacini in 1854, but it was first isolated by Koch in 1883, these organisms are facultative anaerobic, highly mobile, non-spore forming bacteria [2], [3]. On serological bases the most important serovar is O1 which further sub divided into Inaba and Ogawa serogroups [4].

It is generally accepted that eight distinct pandemics of cholera had happened since the onset of the first pandemic in 1817 in Bangladesh [5]. Previously the disease swept the world in six great pandemics (1817 –

1923), and later receded in to its ancestral home in the Indian-Pakistan subcontinent and subsequently in other areas of the world which caused by classical biotype [6].

In 1991 the seventh pandemic strain hit most of the western hemisphere, and the epidemic continued through 1993 by *Vibrio cholerae* O139, studies surveillance during 1996 and 1997 has shown the *Vibrio cholerae* O139 continues to cause cholera outbreaks in India and Bangladesh and coexists with the El-Tor *Vibrio*, this may represent the eighth pandemic [7].

In 1965 – 1966 the El-tor biotype was transmitted from Asia and Middle East to Iraq and Iran through trading in the seventh pandemic [8]. According to several investigators, the presence of cholera in Iraq was due to its warm climate [9- 11].

During the past years, serious outbreaks have occurred in different parts of Iraq, sometimes involving antibiotic resistance strains; many investigators had studied cholera in Iraq provinces with varying subjects [12]. There had been 718 cases of cholera in 2002 and 560 cases in 2001. The incidence of this disease reached its peak in 1998 (2560 cases) [13]. In the first half of 2003, in the southern Al-Basra province, further incidents of cholera had been recorded [13]. Cholera disease continues afflicting Iraqi people thereafter, with variable casualties till the current epidemics.

2. Aim of the study

1. To study the prevalence of cholera infection in different governorates of Iraq during the period from 1/1 to 9/12 / 2015
2. to clarify the governorates with the highest cholera incidence, and try to explain the factors behind this incidence if present

3. Subjects, materials and methods

3.1 Patients groups settings

This is a comparative epidemiological study comprised of 2866 subjects out of 3547 examined cases from different parts of Iraq, who were diagnosed with cholera infection, and their stool samples were sent to the public health laboratory in Baghdad for confirmation. These cholera patients were collected during the period from January to December 2015. Bacteriology, serology and all other lab investigations were worked out in the central health laboratory in Baghdad.

3.2 methods for the laboratory isolation of cholera

A. sampling

1. a small quantity of stool in a wide mouth clean bottle was placed in alkaline peptone water
2. Fecal (stool) specimens were collected in the early stages of the diarrheal disease, When pathogens are present in their highest number, and preferably before antimicrobial treatment is started
3. cotton-tipped sterile rectal swabs were placed in an empty sterile tube with cotton plugs in transport media and sent to public health lab

B. Isolation and identification of vibrio

1. Cholera were grown on a variety of commonly used agar media
2. specimens were enriched at first in APW to enhance the isolation of *vibrio cholera* by increasing their number prior to sub culturing on TCBS
3. after inoculation in APW broths, the specimens were incubated at 35-37 C^{o} for 6-8 hours
4. After incubation, specimens were sub-cultured on TCBS (thiosulfate citrate bile salts sucrose agar)

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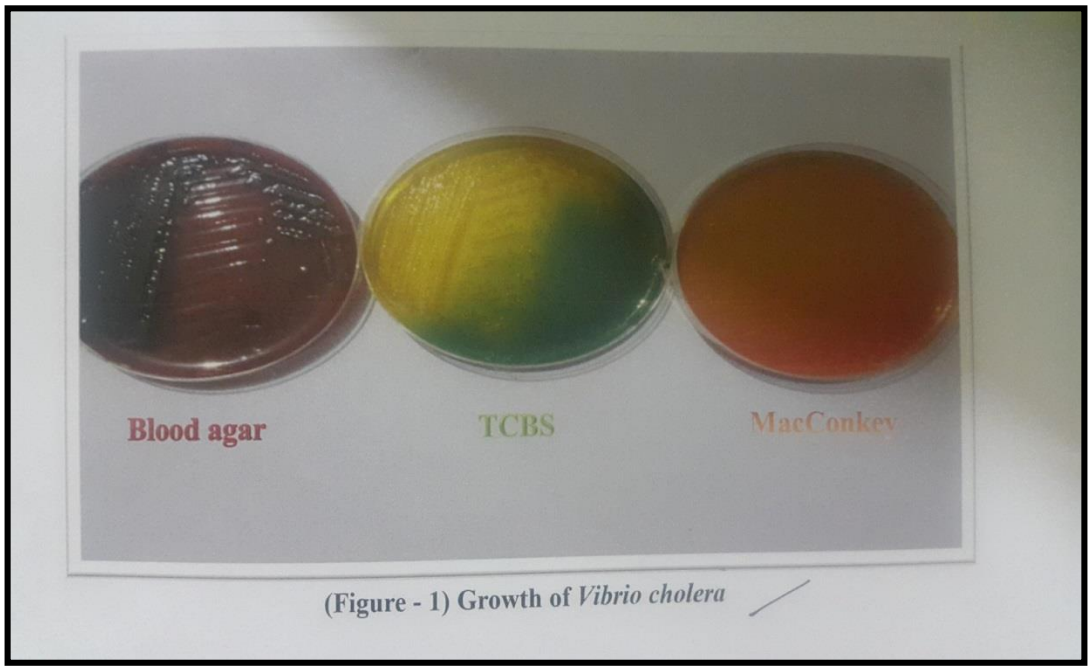
which is the Selective agar medium of choice and on macConkey agar and blood agar.

5. the agar plates were incubated for 18-24 hours at 35-37 Cº

C. identification of vibrio cholera

1. Vibrio. Strains grow as pale, non-lactose –fermenting colonies on macConkey agar.
2. On TCBS agar V. cholera grows as medium-sized convex, smooth, yellow colonies
3. on Blood agar the colonies show Beta hemolysis due to heamolysin enzyme activity



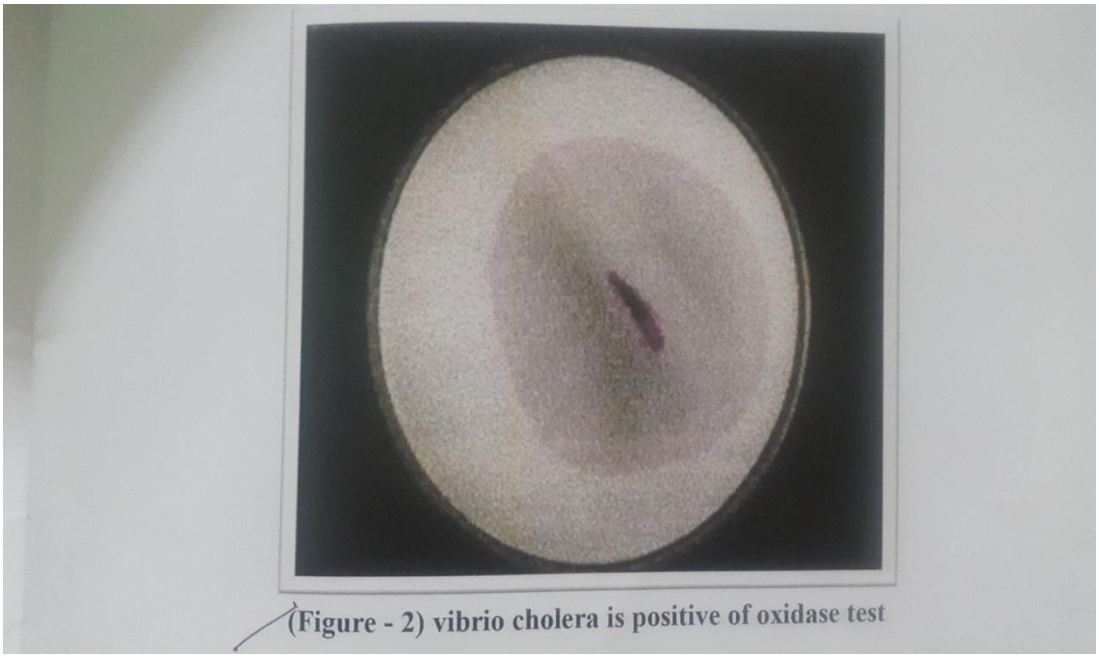
(Figure - 1) Growth of *Vibrio cholera*

D. gram staining

1. samples examined overnight from any media by Gram stain demonstrated typical small, curved gram-negative rods

E. Biochemical identification using oxidase test

1. two to three drops of the oxidase reagent (1%tetramethyl –paraphenylenediamine) were placed on a piece of filter paper in a petri dish.
2. A small amount of fresh growth from the MacConkey or nutrient agar was picked up (Not from TCBS) with a platinum loop and was smeared across the moistened part of the filter paper.
3. A positive reaction was obtained by the appearance of a dark purple color on the paper within 10 Seconds



(Figure - 2) vibrio cholera is positive of oxidase test

3.3 Instruments and Materials

Instrument	Materials
Incubator	Alkaline Peptone Water Enrichment Media(APW)
Microscope	TCBS Selective Agar Media
Wooden Stick	MacConkey Agar
Filter Paper	Blood Agar
Loop	Oxidase Reagent
Refrigerator	Sodium Deoxycholate
Screw Cups	Muller-Hinton Agar
Sterile Swabs	Gram stain kit
Clean cups	Api 20 E Kit
Cary-Blair Transport Media Swabs	

3.4 Statistical analyses

The results of this study were translated into a computerized database structure. Statistical analysis was carried out using the SPSS 17 statistical package. The statistical significance of such associations was assessed by Chi-square (c2) test. Differences were considered statistically significant at $P < 0.05$.

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4. Results and discussion

table (1) Frequency distribution and percentage of total cholera cases in different parts of Iraq with comparisons' significance

Governorate		Patients (n=2866)	100% of total
	Baghdad /AlKarkh	357	12.5%
	Baghdad / Al Rusafa	627	21.9%
	AL Najaf	46	1.6%
	AL – Diwanyia	445	15.5%
	AL-Hilla	675	23.6%
	AL-Muthana	290	10.1%
	Al-Basra	105	3.7%
	Misan	21	0.7%
	Karbala	158	5.5%
	Wasat	68	2.4%
	Al-Nasiryiah	21	0.7%
	Diyala	3	0.1%
	Al-Sulaimanyiah	2	0.1%
	Irbil	10	0.3%
	Salahaldin	2	0.1%
	Duhuk	16	0.6%
	Kirkuk	19	0.7%
	Ninawa	1	0.0%
	P-value	0.000 *	

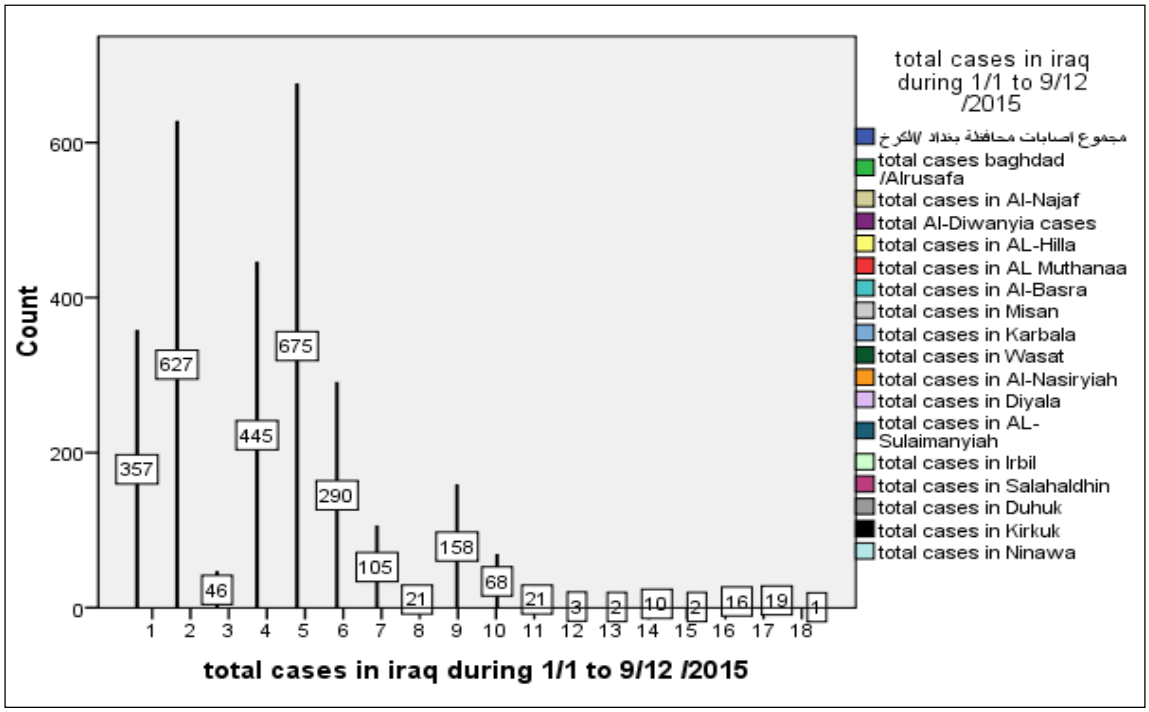

Figure (1) clustered bar chart of total cholera cases distributed among different parts of Iraq during 2015

Table and figure (1) showed the frequency and distribution of total cholera cases in Iraq for the year 2015 with significant difference. total cases had reached 2866 confirmed cholera infections with five Iraqi governorates showed the highest frequency and percentages of total cases; Baghdad AL-Karkh (n=357,12.5%), Baghdad Al-Rusafa (n=627, 21.9%), Al-Hilla (n=675, 23.6%), Al-Diwanyia (n=445, 15.5%) and Al-Muthana (n=290, 10.1%) as shown in the table and figure above.

This high concentration of cholera cases in middle and southern Iraq can be attributed to the fact that the middle and southern parts of Iraq are exposed to a series of serious drought-related problems. This is mainly linked to the absence of applied international law for water distribution in the Euphrates and Tigris rivers between Turkey, Syria, and Iraq, in addition to climate change and unawareness of the water resource problems for more than three decades [126].

Iraq faces a crisis of severe drought particularly in its middle and southern regions. This is the consequence of three decades of war and neglect affecting the environment and natural water resources, thus making it difficult to provide safe drinking or irrigation water for the majority of Iraq's 33 million population by the year 2015 [127], [128].

Moreover, as it was well documented that epidemics usually follows natural disasters such as floods and drought [129], and as it was mentioned earlier that Connections between weather, climate and diseases are well established [66], with many diseases occurring during certain seasons or erupting from unseasonable flood or drought conditions [67]. In addition, several investigators had pointed out to the warm climate of Iraq as a major factor for the presence of cholera in Iraq [9- 11].

In addition, it is important to mention that the availability of safe water and sanitation facilities, the degree of crowding, the underlying health status of the population, and the availability of healthcare services all interact within the context of the local disease ecology to influence the risk for communicable diseases and death in the affected population [129].

Iraq's geography exposes inhabitants to significant threats of waterborne and infectious diseases because of the construction of cities along the Tigris and Euphrates rivers and the proximity to neighboring territories that have frequent disease outbreaks. In the mid-20th century, during a break between World War II and the Persian Gulf wars, Iraqis enjoyed comprehensive health care services, including modern hospitals, throughout the country. By 1992, most hospitals were operating at a fraction of their previous level and faced severe medical and supply shortages. Health care access declined and disease proliferated. Malaria, cholera, gastrointestinal diseases, and typhoid fever became prevalent throughout Iraq [130].

Another effect of war in Iraq is infrastructure deterioration. The destruction of Iraq's water supply system caused widespread failure of water purification and sewage systems [131]. Iraq has become a developing country whose people suffer decreased access to clean water, increased prevalence of malnutrition, and heightened severity of disease outbreaks.

Collectively, these factors might explain to some extent the high incidence of cholera infections in middle and southern Iraqi parts in particular.

5. Conclusions

There was high cholera casualties' proportion in Baghdad / Al Rusafa compared to Baghdad / Alkarkh districts and other parts of Iraq. Al-Hilla city is the Iraqi town with the highest percentage of cholera infections reported compared to other parts of Iraq, followed by Al-Diwanyia which ranks as the second Iraqi city with the highest rate of cholera infections. it is obvious after reading results, that the map of casualties distributes in the cities of middle Euphrates valley and to a lesser extent in south Iraq compared to other parts of Iraq.

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