

## Vibrio cholerae and Changing of Microbial Resistance Patterns in Sistan and Balouchestan Province

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Article information	Abstract
<p>Article history: Received: 25 Non 2011 Accepted: 31 May 2012 Available online: 19 June 2012</p>	<p><b>Background:</b> Cholera is a diarrheal disease in tropical regions that exclusively affects humans. This study conducted to compare changes in antibiotic resistance patterns in 2011 with that of the period of 2008-2010 on cholera patients in the area covered by Zahedan University of Medical Sciences.</p> <p><b>Materials and Methods:</b> In this descriptive study, rectal swab samples within Blair working environment of all suspected patients of each region were sent to the laboratory of each region and diagnostic procedures were performed, and Antimicrobial Susceptibility Testing (AST) was performed through distribution methods on agar disk. Disks were prepared from Iranian Padtan Teb Company and the results were evaluated and reported using NCCLS table and the other experimental antimicrobial susceptibility determination tables.</p> <p><b>Results:</b> In this study, antimicrobial susceptibility testing was performed on 81 positive samples including Ogawa or Inaba during 4 years. The samples in 2008, 2009 and 2010 followed a similar susceptibility pattern and all these samples were resistant to cotrimoxazole, nalidixic acid and ampicillin, had intermediate sensitivity to furazolidone, were sensitive to tetracycline, ciprofloxacin, doxycycline, erythromycin and susceptibility pattern of cases in 2011 included sensitivity to erythromycin and ciprofloxacin and have shown no sensitivity to doxycycline and tetracycline and halo of their lack of growth were placed in the intermediate range.</p> <p><b>Conclusion:</b> Vast border between the province and Afghanistan and Pakistan and inevitability of the occurrence of regional cholera epidemics may cause a serious problem in control of the possible epidemics and recalls the need to perform drug sensitivity tests during epidemics before any remedial action more than ever.</p> <p>Copyright © 2012 Zahedan University of Medical Sciences. All rights reserved.</p>
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### Introduction

Cholerae is a diarrheal disease in tropical areas that is caused by a bacterium called *Vibrio cholerae*, which exclusively affects humans and its symptoms are caused by toxins excreted by vibrio in human intestine [1]. *Vibrio* is one of the four genera of Vibrionaceae family and a number of species of this genus cause cholera. The most important species of this genus is *Vibrio cholerae* in terms of pathogenic which is a curved, gram negative, aerobic, and motile microbe with a polar sequence or flagella. Length of vibrio is about 2-4 microns and they have no spores [2].

Cholera is transmitted fecal-orally. Since more than one million organisms are required to cause the disease, cholera is transmitted almost exclusively through contaminated food or water. Transmission by person to person direct contact, such as contact with patient, is rare. Disease transmission is possible via contaminated hands, contact with fecal materials or patients' vomit and infected bedding and accessories, as well as water contaminated with feces, contaminated or contaminated

water washed raw vegetables and fruits or food contaminated by hands or flies. This bacterium affects the human gastrointestinal mucosa and increases fluid excretion through feces. The incubation period of cholera varies from a few hours to 5 days and is typically 1 to 2 days and symptoms take 2 to 3 days and in some patients, they continue to 5 days. Severity of symptoms depends on the speed and duration of loss of body fluids. The most common way of transmission of cholera epidemic in the country is carrier people and people affected by this disease.

Signs and symptoms of the disease have a wide range. Almost 75% of people who are infected with *Vibrio cholera*, have no symptoms of disease, the other 20% are suffering from diarrhea, which cannot be distinguished from diarrhea caused by other organisms. In 2 to 5% of patients, watery diarrhea, vomiting and dehydration occur. In some cases, even up to 25 liters of water a day is excreted. If this lost water does not compensate for, it will be dangerous and deadly for the individual. Symptomatic

cholera starts with massive feverless watery diarrhea and abdominal tenesmus. This disease is highly contagious. The specific characteristic of cholera is sudden onset of leaping watery diarrhea without pressure that is followed by vomiting, rapid dehydration, cramps and reduced urination. If the body fluids and electrolytes are not quickly replaced, fatality rate of disease will be 30 to 40%. Cholera can be successfully treated with oral or intravenous medical fluid and electrolyte replacement. People's education regarding food and clean water and washing hands after using toilet prevents from the occurrence of new cases [2].

Cholera patients' stool looks like a transparent liquid stained with white mucus, which is called rice-water stool. It has usually no smell or fish odor [1]. Every diarrhea patient from whose stool *Vibrio cholerae* O<sub>1</sub> or O<sub>139</sub> are isolated by valid laboratories will be approved [3]. It should be noted that only groups O<sub>1</sub> and O<sub>139</sub> are known as factors of cholera epidemics. *Vibrio Eltor* is not a separate strain, but it is a biotype of *Vibrio cholerae* which is able to cause cholera like *Vibrio cholerae*, but it is different in terms of biochemical and epidemiological properties. *Vibrio cholerae* is also referred to as classic. According to the characteristics of antigen O which is a body antigen, *vibrio cholerae* is classified into two groups. Those which respond to antiserum O are called *V. cholerae* O<sub>1</sub> and those which do not respond to antiserum O are called *V. cholerae* non O<sub>1</sub>. Another name for this group is Non agglutination vibrios O<sub>1</sub> which is remembered by NAGS. This group includes a group of *Vibrio cholerae* which is not similar to 138 known serogroups of *vibrio cholerae* and is called *vibrio cholerae* O<sub>139</sub> Bengal or new serogroup of *vibrio cholerae*. This group does not result in agglutination, neither with O<sub>1</sub> polyvalent antiserum, nor with monoclonal antibodies, including Ogawa, Inaba and Hikojima. We must know that in addition to the above-mentioned group-specific antigens, *Vibrio cholerae* has type-specific antigens. Thereby, *Vibrio cholerae* will be divided into three types of Ogawa, Inaba and Hikojima [1, 3].

## Materials and Methods

In this descriptive study which includes samples of 2008 to 2011 from all patients with cholera in the areas covered by Zahedan University of Medical Sciences, rectal swab samples within the Blair working environment for all suspected patients were sent to the laboratory in each region and following measures were conducted respectively. That is, first, rectal swab containing sample was transferred to alkaline peptone water medium and was kept at temperature of 37°C for 5-8 h. Simultaneously with this action, initial stool or rectal swab were directly cultured on selected TCBS medium. After keeping peptone water in otto for 5 to 8 hours, the upper part of this medium was cultured by loop and the culture was performed in another TCBS medium. After passing about a night, in the next morning, first and second TCBS mediums were investigated in terms of the presence of suspected colonies and in case of growth of yellow big

convex colonies, we would get of suspicious of *Vibrio cholerae*. Some types of *Proteus*, *Escherichia coli* and *Cocci* also sometimes create yellow or green colonies on TCBS mediums which were distinguished using KIA and SIM mediums and oxidase tests from *vibrio*. Finally, type determination serotyping test was performed with O<sub>1</sub> polyvalent antisera and Monovalent Ogawa and Inaba of Bahar Afshan Company [1].

Antimicrobial susceptibility testing was performed on all positive cases in any epidemic, including Inaba or Ogawa through disc method on agar and using Mueller Hinton medium and comparing bacterial suspension prepared with a McFarland turbidity standard in accordance with NCCLS guidelines [3]. Assessed antibiotics include cotrimoxazole, furazolidone, tetracycline, nalidixic acid, ciprofloxacin, ampicillin, doxycycline and erythromycin and disks were provided from Iranian Padtan Teb Company. However, inaccuracy of results of disc distribution of erythromycin and doxycycline for *vibrio cholerae* are also mentioned [3]; while, the results of disc distribution have not been considered valid for factors of ciprofloxacin, furazolidone and nalidixic acid and interpretive criteria are used for enterobacteriaceae as tentative zone size standards to screen ciprofloxacin-resistant materials.

In addition, furazolidone and nalidixic acid tests are also interpreted for *Vibrio cholerae* and borderline results are presented in the report of results for health authorities as organisms' resistance to these antimicrobial factors [3]. Other results were evaluated using NCCLS table and other tentative tables of antimicrobial susceptibility determination and were reported in three forms of Sensitive, Intermediate and Resistance [4].

## Results

In this study, microbial susceptibility tests were performed on 81 positive samples (table 1), including Ogawa or Inaba, over 4 years. The samples in 2008, 2009 and 2010 followed a similar susceptibility pattern and all these samples were resistant to cotrimoxazole, nalidixic acid and ampicillin, had intermediate sensitivity to furazolidone, and were sensitive to tetracycline, ciprofloxacin, doxycycline, erythromycin, while susceptibility pattern of 2011 included sensitivity to erythromycin and ciprofloxacin and have shown no sensitivity to doxycycline and tetracycline and halo of their lack of growth were placed in the intermediate range (Table 2).

## Discussion

Cholera is a disease which is annually reported in Sistan and Balouchestan province whose main cause is adjacency and wide border with Afghanistan and Pakistan. In addition, it is very important for the health authorities of the province to be aware of the drug resistance condition, which is expanding across the world, as well as our country.

**Table 1.** Table of statistics of cholera positive cases in 2008-2011 separated by nationality and city

City	Year	1387			1388			1389			1390		
		Iranian	Afghan	Pakistani	Iranian	Afghan	Pakistani	Iranian	Afghan	Pakistani	Iranian	Afghan	Pakistani
Zahedan	-	27	-	-	-	-	-	-	-	-	-	-	9
Chabahar	-	-	-	-	-	-	4	-	-	6	-	-	1
Saravan	-	-	-	6	-	-	-	-	-	6	1	-	6
Sarbaz	-	-	-	9	-	2	6	-	-	3	-	-	-
Konarak	-	-	-	-	-	-	-	-	-	2	-	-	-
Nikshahr	-	-	-	-	-	-	-	-	-	1	-	-	-
Iranshahr	-	2	-	-	-	-	-	-	-	-	-	-	-
Total nationality	-	29	-	15	-	2	10	-	-	18	1	-	16
Total	29			17			10			35			

**Table 2.** Comparison of antimicrobial susceptibility test results in 2008, 2009 and 2010 with 2011

Antimicrobial Agents	Disc content (µg)	Zone inhibitory average (mm)	Results-87	Results-88	Results-89	Results-90
Cotrimoxazole	5	8	Resistance	Resistance	Resistance	Resistance
Furazolidon	100	12	Intermediate	Intermediate	Intermediate	Resistance
Tetracycline	30	23	Sensitive	Sensitive	Sensitive	Intermediate
Nalidixic acid	30	10	Resistance	Resistance	Resistance	Resistance
Ciprofloxazine	5	30	Sensitive	Sensitive	Sensitive	Sensitive
Ampiciline	10	8	Resistance	Resistance	Resistance	Intermediate
Doxycycline	30	21	Sensitive	Sensitive	Sensitive	Intermediate
Erytromycine	15	25	Sensitive	Sensitive	Sensitive	Sensitive

This study shows that strains detected in 2011 have intermediate sensitivity to tetracycline and doxycycline compared to the previous years. This sensitivity to these two drugs has been emphasized by Niknejad et al. in 2005 in Golestan province [5].

In the study of Rahbar et al. in 2005, all isolated strains were resistant to cotrimoxazole, nalidixic acid and furazolidone. However, all strains, most of which were Inaba serotypes, were sensitive to tetracycline, ciprofloxacin, erythromycin and ampicillin [4].

In the study of Keramat et al. in 2005, the sensitivity of the two drugs of tetracycline and doxycycline to serotypes Ogawa was emphasized [6]. In another study in 1998, Keramat reported the highest resistance to trimoxazole and furazolidone and the highest sensitivity to ciprofloxacin, nalidixic acid, tobramycin and doxycycline [7]. Moreover, Afzali et al. in 2000 in Kashan reported the diagnosed antibiotic susceptibility of *Vibrio cholerae* to ciprofloxacin, doxycycline, ceftizoxime, cotrimoxazole, nalidixic acid, furazolidone, cephalotin, cephalixin and tetracycline [8]. A study in Calcutta, India in 2008, showed decreased susceptibility to ciprofloxacin and resistance to ampicillin, cotrimoxazole, furazolidone, nalidixic acid and streptomycin [9]. Another molecular study in 2006 in Ghana also emphasized trimoxazole resistance and has stressed that this drug should not be used to treat cholera [10].

In the World Health Organization survey in 2004 and 2005 outbreaks in Cameroon, all strains were sensitive to tetracycline and cotrimoxazole was reported ineffective. In addition, ampicillin resistance was revealed at the end of 2004 and it has been emphasized that these patterns depend on several factors such as age and gender of patients and geographic origin of the disease caused strain [11].

In a study, conducted by Kumar et al. in Bangalore, India, on 730 samples of *Vibrio cholerae* and published in 2009, the frequency of resistance to ampicillin, streptomycin, and penicillin has been reported over 80%, which indicates the increased resistance and changing microbial resistance pattern in recent years [12].

Regardless of isolated serotypes, various reports in the country, during different epidemics in the years before 2011, have almost followed the same antibiotic sensitivity pattern which has changed only in 2011. Especially, the two drugs of tetracycline and doxycycline, which every year have helped health team control the epidemics as sensitive antibiotics; have turned into intermediate sensitive antibiotics. Although this is not considered so much, according to the widespread border of the province with Afghanistan and Pakistan and inevitability of the occurrence of epidemics of cholera in the region, controlling the possible epidemics may be encountered with serious problems and recall the need to perform drug sensitivity tests during the epidemic before any treatment.

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### Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

### Conflict of Interest

The authors declare no conflict of interest.

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