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Antibiotic Resistance of *Vibrio cholerae* Isolates from Kashan, Iran

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ABSTRACT

Aims Cholera is an acute diarrheal disease that can lead to severe dehydration and death. Antibiotic resistance is a big challenge in infective disease like Cholera. The present study aimed to understand the characteristics and trends of antibiotic resistance of *V. cholerae* isolations in and around Kashan, Iran.

Instrument & Methods In this descriptive cross-sectional study, samples were gathered using census method from 1998 to 2013 in Kashan, Iran. 1132 fecal samples of patients with acute diarrhea and 237 samples of suspected water samples were taken. The serotypes and biotypes were determined by an enzymatic method. Antibiotic susceptibility test was performed by using Disk Diffusion Method. Data were analyzed using SPSS 23 software. Fisher-exact and Chi-square tests were used to compare the statistical parameters.

Findings 96 fecal samples (8.5%) and 18 water samples (7.6%) were positive for *Vibrio cholerae*. Non-agglutinating (Nag) isolates (75.4%) were more common than serotype Inaba (13.2%) and Ogawa (11.4%). Nag serotypes were mostly resistant to cefixime (44%) and ampicillin (33%). In contaminated water samples also the most frequent cases were Nag serotype (50%). Nag serotype showed 22.2% of resistance to ampicillin and nitrofurantoin.

Conclusion *Vibrio cholerae* isolates in Kashan, Iran, are highly resistant to antibiotics, especially Nag serotypes.

Keywords *Vibrio cholerae*; Drug Resistance; Serotyping; Multiple Drug Resistance

CITATION LINKS

[1] Trend of antibiotic resistance of Vibrio cholerae strains from East Delhi **[2]** Harrison's Principle of Internal Medicine **[3]** Epidemioloic and drug Resistant Pattern of Vibrio Cholerae O1 Biotype EL Tor, Serotype Inaba during the summer of 2005 outbreak in Iran **[4]** A localised outbreak of Vibrio cholerae O139 in Kolkata, West Bengal **[5]** Survival of classic cholera in Bangladesh **[6]** Freshwater non-O1 Vibrio cholerae infection **[7]** Clinical and Epidemiologic Features of Diarrheal Disease due to Aeromonashydrophila and Plesiomonasshigelloides Infections Compared with Those due to Vibrio cholerae Non-O1 and Vibrio parahaemolyticus in Bangladesh **[8]** A recent outbreak of cholera due to Vibrio cholerae O1 Ogawa in & around Chandigarh, North India **[9]** Epidemiology &antibiograms of Vibrio cholerae isolates from a tertiary care hospital in Chandigarh, north India **[10]** Multiple antibiotic resistance of Vibrio choleraeserogroup O139 in China from 1993 to 2009 **[11]** Multi-drug resistant toxigenic Vibrio cholerae O1 is persistent in water sources in New Bell-Douala, Cameroon **[12]** Evaluation of antibiotic sensitivity of Shigella, Salmonella, and Vibrio Cholera in patients with acute diarrhea referred to reference laboratory of Kashan University of Medical Sciences from 2000 to 2001 **[13]** Antibiotic resistance and plasmid profile of environmental isolates of Vibrio species from Mai Po Nature Reserve, Hong Kong **[14]** Changing profile of Vibrio cholerae O1, O139 in Delhi & its periphery (2003-2005) **[15]** Molecular epidemiology of Vibrio cholerae causing outbreaks & sporadic cholera in northern India **[16]** Antibiotic resistance mechanisms of Vibrio cholerae **[17]** Detection and molecular characterization of Vibrio vulnificus from coastal waters of Malaysia **[18]** Vibrio cholerae 01 ogawa (eitor) diarrhoea at sevagram **[19]** Identification of environmental plasmid-bearing Vibrio species isolated from polluted and pristine marine reserves of Hong Kong, and resistance to antibiotics and mercury **[20]** Genetic determinants of virulence, antibiogram and altered biotype among the Vibrio cholerae O1 isolates from different cholera outbreaks in India **[21]** Genetic diversity and virulence potential of environmental Vibrio cholerae population in a cholera-endemic area **[22]** Cholera epidemic in and around Kolkata, India: Endemicity and management **[23]** Multiple antibiotic resistance profiles of Vibrio cholerae non-O1 and non-O139

Introduction

Cholera is an acute diarrheal disease that can lead to severe and rapidly progressive dehydration in hours and death. The epidemic spread of cholera from the Asia to rest of the world attributed to increasing number of trips [1]. Drinking water contaminated with human feces is the most common way of getting cholera. Clinical symptoms include watery diarrhea, vomiting, tachycardia, weak pulse, orthostatic hypotension, somnolence, and eventually coma [2, 3].

Two serogroups of *Vibrio cholerae*, O1 and O139, cause outbreaks of cholera. O1 causes the majority of outbreaks, while O139 first was identified in Bangladesh in 1992 and has been confined to South-East Asia. V. cholera O1 has 2 biotypes, classical and El Tor, and each biotype has 2 distinct serotypes, Inaba and Ogawa [4, 5]. *V. cholerae* is best recovered from stool specimens using thiosulfate-citrate-bile salt-sucrose (TCBS) agar where the organisms are easily identified by their characteristic yellow colonies. Isolates can be further characterized through using the classic *V. cholerae* strain agglutinates with type O1 antiserum. Organisms that are identified biochemically as *V. cholerae* but do not agglutinate with O1-antiserum are designated V. cholerae non-O1 serotype or NAG (non-agglutinable) *V. cholera* [6]. NAG organisms rarely make the classic *V. cholerae* enterotoxin, but they may make other enterotoxins, cytotoxins, and hemolysins. Many articles have reviewed the world's literature and reported 23 cases of sepsis from NAG infection with a 61.5% mortality [7].

Antibiotic therapy decreases cholera duration and fluid loss volume and accelerate the clearance of the organism in the feces. Like other infective disease, abundant antibiotic application has been leaded in drug resistance [8]. Different trends of antibiotic resistance of *V. cholerae* have been observed around the world [1, 9-11]. In the study performed in Iran by Rahbar *et al*., all strains of *V. cholerae* were resistant to cotrimoxazole, nalidixic-acid and furazolidone and were sensitive to tetracycline, ciprofloxacin, erythromycin and ampicillin [3]. Afzali *et al*. have shown that the sensitivity of cholera to ciprofloxacin, doxycycline and tetracycline was 100% and to cotrimoxazole and nalidixic-acid was 87.5% and 83.5%, respectively [12]. In another study performed in Hong Kong, all three strains of *V. cholerae* (1, 2, 3mp) were highly resistant to the B-lactamase family and were sensitive to tetracycline, chloramphenicol, nalidixic-acid and streptomycin [13]. Considering the different patterns of sensitivity and resistance in *V. cholerae*, prescription of proper antibiotics to treat patients help to prevent drug resistance and reduce the costs [14]. Continuous monitoring and evaluation of antibiotic resistance in various regions can help researchers and medical supervisors to control and prevent re-epidemic of *V. cholera* [15].

The present study aimed to understand the characteristics and trends of antibiotic resistance of *V. cholerae* isolations in and around Kashan, Iran.

Instrument & Methods

In this descriptive cross-sectional study, samples were gathered using census method from 1998 to 2013 in Kashan, Iran. 1132 fecal samples (rectal swab) of patients with acute diarrhea who referred to health centers, hospitals and private clinics, and 237 samples of suspected water samples were prepared and transferred to Cary Blair transport medium.

The samples were transferred to alkaline peptone water medium until the pH reached to 8.8. Samples were incubated in Thiosulfate Citrate Bile Sucrose (TCBS) for 24 hours. The serotypes and biotypes were determined by an enzymatic method. Antibiotic susceptibility test was performed by using Disk Diffusion Method (Kirby Bauer). Each isolates was tested against eight antibiotics: Ciprofloxacin (5µg), Ampicillin (10µg), Cotrimoxazol (25µg), Doxycycline (30µg), Tetracycline (30µg), Nitrofurantoin (300µg), Cefixime (5µg) and Erythromycin (15µg). Standard strains of *Escherichia coli* (ATCC 25922) was used as control strains.

Water suspected to contamination with cholera was transferred to holder machine and after passing through the filter, cultured in TCBS medium and the antibiogram has been conducted. To better justification and interpretation, the results were classified based on some variables like sources, year and patients age. Study was approved by the ethics committee of the research deputy in Kashan University of Medical Sciences.

Data were analyzed using SPSS 23 software. Fisher-exact (to compare antibiotic resistance with water and patient sample) and Chi-square (to analyze the relationship of antibiotic resistance with age and sex) tests were used to analyze data.

Findings

Totally 96 fecal samples (8.5%) and 18 water samples (7.6%) were positive for *Vibrio cholerae*. 50 cases (47.3%) of 96 patients were male and 46 cases (52.6%) were female (p>0.05). 23 cases (21%) were less than 15 year-old and 73 cases (78.9%) were above 15 year-old. Antibiotic resistance was more in the age group above 15 year-old in all antibiotics except ciprofloxacin, doxycycline, and tetracycline (Figure 1). 32 samples (33.3%) were recorded before 2008. No cases have been reported in 2010 and 2011. Resistance to all antibiotics has been increased over 15 years, especially to nitrofurantoin and cefixime.

Non-agglutinating (Nag) isolates (75.4%) were more common than serotype Inaba (13.2%) and Ogawa (11.4%). Less antibiotic resistance was observed in isolated samples of contaminated water and they were only resistant to 4 antibiotics; ampicillin, nitrofurantoin, cotrimoxazole and cefixime. However, fecal isolates were resistant to all antibiotics; maximum resistance to cefixime (37.5%) and the least resistance to ciprofloxacin 1.1%. No significant differences were observed between two groups of patients and contaminated water in antibiotic resistance (p>0.05).

Figure 1) Comparison the frequency (Numbers in parentheses are pernectages) of the antibiotic resistance based on the sources and age of samples by Fisher Exact test

Inhibition type	Source		p Value	Age		p Value
	Patient sample	Water sample		15 <	15 ≥	
Ciprofloxacin						
Susceptible	90 (93.7)	18 (100)		69 (71.8)	21 (21.8)	
Intermediate	5 (5.2)	0	0.999	4 (4.1)	1 (1)	0.5
Resistant	1 (1.1)	0		0	1 (1)	
Ampicillin						
Susceptible	31 (32.2)	5 (27.7)		21 (21.8)	10 (10.4)	
Intermediate	30 (31.2)	8 (44.4)	0.999	24 (25)	6 (6.2)	0.999
Resistant	35 (36.4)	5 (27.7)		28 (29.1)	7 (7.2)	
Cotrimoxazol						
Susceptible	61 (63.5)	13 (72.2)		44 (45.8)	17 (17.7)	
Intermediate	16 (16.6)	4 (22.2)	0.3	12 (12.5)	4 (4.1)	0.999
Resistant	19 (19.7)	1 (5.5)		17 (17.7)	2 (2)	
Doxycycline						
Susceptible	87 (90.6)	17 (94.4)		67 (69.7)	20 (20.8)	
Intermediate	6 (6.2)	1 (5.5)	0.999	5 (5.2)	1 (1)	0.4
Resistant	3(3.1)	0		1 (1)	2 (2)	
Tetracycline						
Susceptible	81 (84.3)	16 (88.8)		63 (65.6)	18 (18.7)	
Intermediate	12 (12.5)	2 (11.1)	0.999	9 (9.3)	3 (3.1)	0.5
Resistant	3 (3.1)	0		1 (1)	2 (2)	
Nitrofurantoin						
Susceptible	59 (61.4)	13 (72.2)		49 (51)	10 (10.4)	
Intermediate	13 (13.5)	2 (11.1)	0.5	9 (9.3)	4 (4.1)	0.5
Resistant	24 (25)	3 (16.6)		15 (15.6)	9 (9.3)	
Cefixime						
Susceptible	52 (54.1)	15 (83.3)		44 (45.8)	8 (8.3)	
Intermediate	8 (8.3)	1 (5.5)	0.2	2 (2)	6 (6.2)	0.01
Resistant	36 (37.5)	2 (11.1)		27 (28.1)	9 (9.3)	
Erythromycin						
Susceptible	50 (52)	6 (33.3)		35 (36.4)	15 (15.6)	
Intermediate	44 (45.8)	12 (66.6)	0.999	37 (38.5)	7 (7.2)	0.07
Resistant	2 (2)	0		1 (1)	1 (1)	

In fecal samples, 77 isolates (80.2%) were Nag serotype, which had the highest level of antibiotic resistance. Nag serotypes were mostly resistant to cefixime (44%) and ampicillin (33%). Ampicillin (32%) got the most resistance in Ogawa serotypes, and nitrofurantoin and cotrimoxazol (20%) in Inaba serotype. In contaminated water samples, also the most frequent cases were Nag serotype (50%). Nag serotype showed 22.2% of resistance to ampicillin and nitrofurantoin. It showed intermediate resistance to all antibiotics except ciprofloxacin and erythromycin. Ampicillin and cefixime showed the highest resistance in Ogawa serotype (50%). Inaba serotype showed no antibiotic resistance in these samples.

Discussion

The aim of this study was to evaluate the antibiotic resistance of *V. cholerae* isolates in Kashan, Iran. The results confirm increasing problem of bacterial resistance to antibiotics in patients by careless consumption or administration of antibiotics. As it can be seen in literature, various resistance mechanisms such as efflux pumps, spontaneous chromosomal mutation, conjugative plasmids, SXT elements and integrons are involved in *V. cholerae* resistance [16].

In a cholera outbreak in Iran in 2005, it was reported that antibiotic resistance to cotrimoxazol, nalidixic acid and furazolidone were 100 %, whereas antibiotic sensitivity to tetracycline, ciprofloxacin, erythromycin and ampicillin were 100 % [3]. While Afzali *et al.* have shown antibiotic sensitivity of *V. cholerae* to ciprofloxacin, doxycycline, and tetracycline (100%), ceftizoxim (95.8%) , cotrimoxazol (87.5%) , nalidixic-acid (83.3%) , and ampicillin (62.5%) [12]. In another study in Malaysia, have reported that all strains isolated from coastal waters were sensitive to cotrimoxazol, erythromycin and ciprofloxacin and most antibiotic resistance was to doxycycline [17]. In the present study, the highest antibiotic resistance was for Nag serotype, whereas the lowest was for Inaba serotype which was 100% sensitive to all antibiotics. Many studies, like Wang *et al.* [13], Batra *et al.* [18] and Zhang *et al.* [19] have demonstrated that generally antibiotic resistance in Inaba serotype is lower than other serotypes.

Increasing body of evidences demonstrate that antibiotic therapy has increased antibiotic resistance of *V. cholerae* dramatically [9, 20], which was reached from 1 antibiotic in 1998 to 3 antibiotics in 2004. Similar to current study, some previous researches indicate on increasing rate of antibiotic resistance during years. Faruque *et al.*, for example report at the beginning of 1998 antibiotic resistance of cotrimoxazole (0%), tetracycline (2.5%), and ciprofloxacin (4.6%) reached to 10.2%, 13.6% and 12.4% in 2004, respectively [21]. No significant differences were observed in the antibiotic resistance among sexes in the present study, however, other studies reported a significant higher resistance among men than women [22, 23].

The results of this study reveal that resistance increases among selective antibiotics for treatment of *V. cholerae* in recent years, which can be dangerous for treatment due to the emergence of multidrug resistance strains. Therefore, frequent monitoring and surveillance of cholera outbreak is useful. The increase in antibiotic resistance among strains isolated from the environment should be considered.

Conclusion

Vibrio cholerae isolates in Kashan, Iran, are highly resistant to antibiotics, especially Nag serotypes.

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