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A systematic review and meta-analysis on the epidemiology of antibiotic resistance of *Vibrio cholerae* in Iran

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Key words: V. cholerae, antibiotic resistance, Iran
Parole chiave: V. cholerae, antibioticoresistenza, Iran

Abstract

Background. *Cholera, an acute diarrheal disease caused by Vibrio cholerae (V. cholerae), is an endemic disease and a major public health problem in Iran. Antibiotic therapy can decrease duration of the disease, transmission of infection and contamination of the environment. Considering different pattern of V. cholerae antibiotic resistance around the world, the aim of the current systematic review and meta-analysis was to evaluate the prevalence of antibiotic resistance of V. cholerae in Iran.*

Methods. *A systematic review of the literature was performed using related keywords in the electronic national and international databases including SID, Irandoc, Iran Medex and Magiran as well as PubMed, Scopus, Google Scholar and ISI web of knowledge. Up to July 31, 2018, 27 eligible papers were included in our meta-analysis based on the defined inclusion criteria.*

Results. *V. cholerae O1 was the most prevalent strain isolated in Iran and exhibited a high resistance rate against numerous antibiotics including chloramphenicol (33.6%), oxytetracycline (40.2%), trimethoprim/sulphamethoxazole (86%), tetracycline (34.5%), furazolidone (69.8%), streptomycin (93.8%), polymyxin (80.7%), ampicillin (32.1%), nalidixic acid (88.9%), kanamycin (29%) and amoxicillin (30.5%).*

Conclusion. *According to the meta-analysis results, antibiotic therapy with ciprofloxacin, doxycycline, erythromycin, gentamicin, azithromycin, cefixime and cefepime could be effective for the treatment of severe cases of cholera in Iran.*

Introduction

Vibrio cholerae (*V. cholerae*) is a curved Gram-negative bacillus which belongs to the family *Vibrionaceae* and was first isolated and described by Koch at the end of the 19th century (1). The bacterium is a causative agent for cholera, an acute intestinal infection that is transmitted by ingestion of contaminated water and food (2). In addition to *V. cholerae*, other important *Vibrio* species in the genus *Vibrio* include *V. parahaemolyticus*, *V. harveyi, V. alginolyticus, V.vulnificus, V. anguillarum* and *V. fluvialis,* which are isolated from fish, shrimp and lobster in aquatic environments and are pathogenic for humans (3). *V. cholerae* can also live in marine and estuarine environments and multiply freely in water for years (1-3). Therefore, the bacterium can

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cause endemic, epidemic and even pandemic diseases, especially where sanitation situation is weak and there is no access to safe water (1-3). According to the latest World Health Organization (WHO) estimation, there are from 1.3 to 4.0 million new cases of cholera causing 21,000 to 143,000 deaths each year in the world (4). Among many serogroups of *V. cholerae*, based on O antigen, O1 and O139 strains of *V. cholerae* are responsible for cholera in humans by secreting an enterotoxin encoded by the *ctx* gene (5). These serogroups are also associated with seven widespread pandemics of cholera and still remain as a worldwide problem in both developed and developing countries, especially in Asia, Africa and Latin America (5). This severe diarrhoeal disease is also a public health problem in Iran and outbreaks occur annually among Iranian people (6). In Iran, cholera is endemic, especially in regions close to the borders, and *V. cholerae* O1 Ogawa and Inaba serogroups of El Tor biotype were reported to be responsible for recent outbreaks (1998–2011) in Iran along with changeable antibiotic-resistant patterns (6-8). *V. cholerae* infection severity can range from mild to moderate in endemic areas (90% of cases), and to severe and rapidly fatal diarrhea (10% of cases) (9). Early diagnosis along with oral rehydration salts (ORS) solution containing glucose, potassium chloride, sodium chloride and trisodium citrate are essential for the successful treatment of cholera accompanied by mild watery diarrhea (9). However, management of severe cholera with severe dehydration requires intravenous rehydration along with appropriate antimicrobial therapy in order to decrease the duration of the disease, the volume of rehydration fluids, the transmission of infection and the contamination of the environment through reducing *V. cholerae* excretion in stool (9). Previously, due to the low resistance rate of *V. cholerae* to commonly used antibiotics, determination of the antibiotic susceptibility of bacteria was not recommended (10). However, reports have shown that resistance rate of *V. cholerae* is increasing worldwide (11). There is no overall estimation of antibacterial resistance of *V. cholerae* in Iran. Hence, this systematic review and meta-analysis was undertaken to determine the pattern of antibacterial resistance of *V. cholerae* isolated from clinical samples in Iran.

Materials and Methods

Data sources and search methods

Up to July 31, 2018, we searched the literature with the purpose of identifying studies reporting the prevalence of antibiotic resistance of *V. cholerae* in Iran. Main searched databases were PubMed, Scopus, Google Scholar and ISI web of knowledge to find eligible published English-language papers, as well as Scientific Information Database (SID), Iranian Research Institute for Information Science and Technology (Irandoc), Iran Medex and Magiran for Persian-language papers. Three main MeSH terms used for database searching were "antibiotic resistance, *V. cholerae*, Iran". We also reviewed references of the included articles to find any relevant studies.

Selection of studies

The search results were assessed by two reviewers independently after review of title, abstract and full text of articles in order to collect eligible studies using inclusion and exclusion criteria. Our inclusion criteria were: cross-sectional studies, articles published in Persian or English languages, and articles that evaluated antibiotic susceptibility patterns of *V. cholerae* collected from clinical samples in Iran. The reasons for our exclusions were: investigation of antibiotic resistance of *Vibrio* species other than *V. cholerae*, investigation of antibiotic resistance of *V. cholerae* in other countries, investigation of antibiotic resistance of *V. cholerae* collected

from environmental samples, investigation of simultaneous resistance to antibiotics, other study types except cross-sectional (e.g. reviews, letters and case report studies), abstract list of congresses and duplicates, and insufficient data.

Data collection

We extracted data from included articles and categorized. As shown in Table 1, required extracted data were: first author's name, year of the study, region of the study, type of sample, bacterial identification methods, number of isolated bacteria, methods used for evaluation of antibiotic resistance, and drug resistance of *V. cholerae* to various antibiotics. The PRISMA (Preferred Reporting Items for Systematic review and Meta-Analyses) statement was used to improve systematic reviews and meta-analyses in all sections of the materials and methods (12).

Analytical approach

To perform meta-analyses, we analyzed the data collected from included studies in the Comprehensive Meta-Analysis (CMA) software version 2.2 (Biostat, Englewood, NJ, USA). A fixed-effect model in low heterogeneity and random effect model in large heterogeneity were applied to calculate the pooled data on the prevalence of *V. cholerae* antibiotic resistance in Iran. *V. cholerae* antibiotic resistance rate in different cities of Iran was calculated and expressed as percentage and 95% confidence intervals (95% CIs).

Cochrane Q-test ($p < 0.05$ was considered statistically significant) and I-squared (I^2) index as well as funnel plots were used to check the possibility of heterogeneity and publication bias, respectively.

Results

Results of the search

A total of 283 articles were collected from PubMed, Scopus, Google Scholar, ISI web of knowledge and Iranian databases. As shown in Figure 1, after reviewing the titles, abstracts and full texts of the articles, and removing duplicates, non-relevant studies, articles with insufficient data, reviews, letters and case reports, 27 relevant articles were

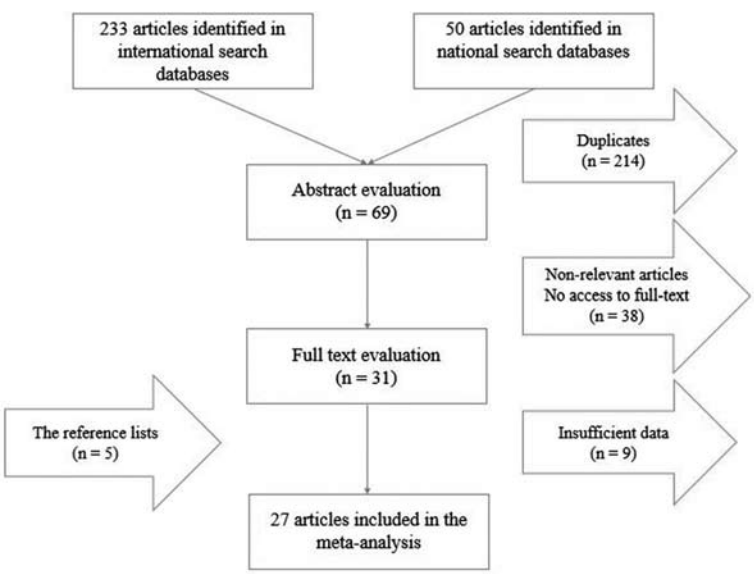

Figure 1 - Flow diagram of the article selection process

Table 1 - Profiles of included studies in the meta-analysis

First author (Ref)	Year	Area	Sample type	Bacterial identification method(s)	Strains (n)	AST	C	CIP	DOX	E	OXT	SXT	T	GM	F	S	PB	AP	AZ	NA	CFM	**CP**	**K**	**AMX**
Adabi et al (13)	2004-2006	*Different provinces	Clinical	**Microbiological techniques	60	Disk diffusion method	43	5	0	37	0	48	12	0	ND	48	ND	6	ND	ND	ND	ND	ND	ND
Rahbar et al (14)	2005	Different provinces	Clinical	Microbiological techniques	100	Disk diffusion method	ND	0	ND	0	ND	100	0	ND	100	ND	ND	0	ND	100	ND	ND	ND	ND
Hajia et al (15)	2011-2013	Different provinces	Clinical	Microbiological techniques	192	E-test	ND	0	ND	0	ND	184	118	ND	ND	ND	ND	0	ND	183	5	ND	ND	ND
Rahmani et al (16)	2005-2007	Different provinces	Clinical	Microbiological techniques	107	Broth microdilution	106	ND	ND	ND	ND	105	ND	ND	ND	97	ND	ND	ND	ND	ND	ND	ND	ND
Masoumi-Asl et al (17)	2013	Different provinces	Clinical	Microbiological techniques	60	E-test	ND	0	ND	14	ND	60	60	ND	ND	ND	ND	0	ND	60	0	ND	ND	ND
Rahbar et al (18)	2008	Different provinces	Clinical	Microbiological techniques	220	Disk diffusion method	*** LD	0	ND	0	ND	220	0	ND	220	ND	ND	ND	ND	220	ND	ND	ND	ND
Khany et al (19)	2013	Different provinces	Clinical	Microbiological techniques	102	E-test	ND	ND	ND	ND	ND	ND	102	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Farzami et al (20)	2013	Different provinces	Clinical	Microbiological techniques	257	E-test	ND	0	ND	0	ND	257	257	ND	ND	ND	ND	0	ND	257	0	ND	ND	ND
Bakhshi et al (21)	2005	Different provinces	Clinical	Microbiological techniques	25	Disk diffusion method	22	0	2	ND	1	23	20	0	ND	23	21	13	ND	ND	ND	ND	ND	ND
Sedaghat et al (22)	2005	Different provinces	Clinical	Microbiological techniques	100	Disk diffusion method	27	0	0	63	62	74	53	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Marashi et al (23)	2004-2010	Different provinces	Clinical	Microbiological techniques	94	Disk diffusion method	ND	ND	ND	ND	ND	92	ND	ND	ND	92	ND	ND	ND	ND	ND	ND	ND	ND
Pourshafie et al (24)	1999-2000	Tehran	Clinical	Microbiological techniques	58	Disk diffusion method	0	0	0	0	58	58	0	0	58	58	ND	ND	ND	ND	ND	ND	ND	ND
Pourshafie et al (7)	2005	Tehran	Clinical	Microbiological techniques	15	Disk diffusion method	10	ND	1	0	ND	9	13	1	0	10	13	4	ND	ND	ND	ND	ND	ND

Sedaghat et al (25)	2002-2003	Tehran	Clinical	Microbiological techniques	32	Disk diffusion method	10	0	2	16	29	32	31	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Barati et al (26)	2011	Alborz	Clinical	Microbiological techniques	239	Disk diffusion method	ND	0	10	13	ND	228	36	ND	202	ND	ND	110	2	ND	ND	ND	ND	ND
Ranjbar et al (27)	2008	Alborz	Clinical	Microbiological techniques	75	Disk diffusion method	ND	ND	24	53	ND	52	13	ND	41	ND	ND	ND	ND	54	5	1	41	46
Pourshafie et al (24)	1999-2000	Khuzestan	Clinical	Microbiological techniques	60	Disk diffusion method	1	0	0	0	57	58	16	0	20	60	ND	ND	ND	ND	ND	ND	ND	ND
Sedaghat et al (25)	2002-2003	Khuzestan	Clinical	Microbiological techniques	30	Disk diffusion method	7	0	3	18	30	30	30	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pourshafie et al (24)	1999-2000	Kerman	Clinical	Microbiological techniques	44	Disk diffusion method	1	0	2	1	1	44	2	0	44	44	ND	ND	ND	ND	ND	ND	ND	ND
Pourshafie et al (24)	1999-2000	Kerman-shah	Clinical	Microbiological techniques	4	Disk diffusion method	0	0	0	0	4	4	0	0	4	4	ND	ND	ND	ND	ND	ND	ND	ND
Sedaghat et al (25)	2002-2003	Kerman-shah	Clinical	Microbiological techniques	12	Disk diffusion method	6	0	0	7	7	4	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pourshafie et al (24)	1999-2000	Kashan	Clinical	Microbiological techniques	29	Disk diffusion method	0	0	0	29	0	0	0	0	0	29	ND	ND	ND	ND	ND	ND	ND	ND
Sedaghat et al (25)	2002-2003	Kashan	Clinical	Microbiological techniques	26	Disk diffusion method	0	0	0	22	0	0	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Afzali et al (28)	1998-2013	Kashan	Clinical	Microbiological techniques	96	Disk diffusion method	ND	1	3	2	ND	19	3	ND	ND	ND	ND	35	ND	ND	36	ND	ND	ND
Afzali et al (29)	2000-2001	Kashan	Clinical	Microbiological techniques	24	Disk diffusion method	0	0	0	ND	ND	6	0	ND	10	ND	ND	ND	ND	7	ND	ND	ND	ND
Afzali et a (30)	1998-2009	Kashan	Clinical	Microbiological techniques	58	Disk diffusion method	ND	1	1	0	ND	11	1	ND	9	ND	ND	18	ND	ND	ND	ND	ND	6
Pourshafie et al (24)	1999-2000	Sistan & Baluchastan	Clinical	Microbiological techniques	5	Disk diffusion method	0	0	0	0	0	5	0	0	5	5	ND	ND	ND	ND	ND	ND	ND	ND

First author (Ref)	Year	Area	Sample type	Bacterial identification method(s)	Strains (n)	AST	C	CIP	DOX	E	OXT	SXT	T	GM	F	S	PB	AP	AZ	NA	CFM	**CP**	**K**	**AMX**
Pourshafie et al (7)	2005	Sistan & Baluchastan	Clinical	Microbiological techniques	8	Disk diffusion method	8	ND	0	0	ND	8	7	0	5	8	6	6	ND	ND	ND	ND	ND	ND
Tabatabaei et al (31)	2013	Sistan & Baluchastan	Clinical	Microbiological techniques	48	Disk diffusion method	ND	0	ND	11	ND	43	29	ND	ND	ND	ND	27	ND	21	ND	ND	ND	ND
Salimi et al (32)	2008-2011	Sistan & Baluchastan	Clinical	Microbiological techniques	81	Disk diffusion method	ND	0	*** LD	0	ND	81	*** LD	ND	*** LD	ND	ND	81	ND	81	ND	ND	ND	ND
Rezaie et al (33)	2012-2013	Sistan & Baluchastan	Clinical	Microbiological techniques	20	Disk diffusion method	0	ND	ND	ND	ND	12	ND	ND	ND	20	ND	ND	ND	ND	ND	ND	ND	ND
Ranjbar et al (34)	2012-2013	Sistan & Baluchastan	Clinical	Microbiological techniques	30	Disk diffusion method	0	0	ND	30	ND	18	15	0	ND	30	ND	0	ND	ND	ND	ND	ND	ND
Pourshafie et al (7)	2005	Golestan	Clinical	Microbiological techniques	14	Disk diffusion method	12	ND	1	2	ND	13	10	0	0	13	10	6	ND	ND	ND	ND	ND	ND
Niknejad et al (35)	2005	Golestan	Clinical	Microbiological techniques	95	Disk diffusion method	38	ND	15	48	15	35	18	ND	48	ND	ND	43	ND	80	ND	ND	ND	ND
Pourshafie et al (7)	2005	Qom	Clinical	Microbiological techniques	13	Disk diffusion method	13	ND	0	0	ND	13	12	0	0	13	11	5	ND	ND	ND	ND	ND	ND
Keramat et al (36)	2005	Hamadan	Clinical	Microbiological techniques	60	Disk diffusion method	ND	5	20	37	ND	39	14	ND	60	ND	ND	ND	ND	ND	ND	ND	7	ND
Keramat et al (36)	1998	Hamadan	Clinical	Microbiological techniques	100	Disk diffusion method	67	8	15	27	ND	100	ND	ND	100	ND	ND	92	ND	2	ND	ND	ND	ND
Talebi et al (37)	1998	Qazvin	Clinical	Microbiological techniques	51	Disk diffusion method	23	ND	25	32	ND	ND	22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Taramsari et al (38)	2011	Guilan	Clinical	Microbiological techniques	142	Disk diffusion method	27	1	12	8	ND	103	23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Abbreviations: C-chloramphenicol; CIP-ciprofloxacin; DOX-doxycycline; E-erythromycin; OXT-oxytetracycline; SXT-trimethoprim/sulphamethoxazole or co-trimoxazole; T-tetracycline; GM-gentamicin; F-furazolidone; S-streptomycin; PB-polymyxin; AP-ampicillin; AZ-azithromycin; NA-nalidixic acid; CFM-cefixime; CP-cefepime; K-kanamycin; AMX-amoxicillin; ND- Not determined; AST-antimicrobial susceptibility testing.
*There was data as total; **Alkaline peptone water, thiosulphate-citrate-bile salt-sucrose agar (TCBS), and standard biochemical tests.
***LD: low data.

selected for the meta-analysis. The main characteristics of 27 included studies are indicated in Table 1.

Included studies

Of the 27 included studies in this review, antibiotic resistance of *V. cholerae* was reported from Tehran (n = 3), Alborz (n = 2), Khuzastan (n = 2), Kerman (n = 1), Kermanshah (n = 2), Kashan (n = 5), Sistan & Baluchastan (n = 6), Golestan (n = 2), Qom (n = 1), Hamadan (n = 2), Qazvin (n = 1), and Guilan (n = 1) provinces. Disk diffusion method, broth microdilution and E-test were the most commonly used techniques to assess the susceptibility of *V. cholerae* isolates. Samples were clinical, and microbiological techniques were used for bacterial identification. Additionally, *V. cholerae* O1 serogroup, Ogawa/Inaba serotype and El Tor biotype were the most frequent strains in the included studies. O1 polyvalent and Ogawa/Inaba monospecific antisera were used to determine specific serogroups. Finally, funnel plot of the meta-analysis was used to check the possibility of publication bias of included studies on antibiotic resistance of *V. cholerae* to each drug (Figure 3). In some antibiotic resistances, the distribution of studies was symmetrical in funnel plot and vice versa.

Characteristics of V. cholerae antibiotic resistance

In the present study, the prevalence of *V. cholerae* resistance to different antibiotics was evaluated. Reported antibiotics were chloramphenicol, ciprofloxacin, doxycycline, erythromycin, oxytetracycline, trimethoprim/sulphamethoxazole, tetracycline, gentamicin, furazolidone, streptomycin, polymyxin B, ampicillin, azithromycin, nalidixic acid, cefixime, cefepime, kanamycin and amoxicillin. The highest and lowest resistance rates for each antibiotic in different provinces are listed in Table 2.

The data presented in the current study indicated a low rate of *V. cholerae* resistance against ciprofloxacin (1.8%), doxycycline (7.2%), erythromycin (18.6%), gentamicin (2.8%), azithromycin (0.8%), cefixime (3.7%) and cefepime (1.3%) in Iran (Table 2). Additionally, the highest rate of *V. cholerae* resistance in Iran were observed against chloramphenicol (33.6%), oxytetracycline (40.2%), trimethoprim/sulphamethoxazole (86%), tetracycline (34.5%), furazolidone (69.8%), streptomycin (93.8%), polymyxin (80.7%), ampicillin (32.1%), nalidixic acid (88.9%), kanamycin (29%) and amoxicillin (30.5%) (Table 2).

Discussion

Insufficient access to safe water and food along with other factors including age and climatic factors (e.g. temperature and humidity), are the major risk factors for the incidence of cholera infection (17, 39). In Iran, in addition to the above mentioned risk factors, the arrival of Afghan and Pakistani immigrants from ungovernable border crossing in eastern provinces especially Sistan & Baluchastan plays another key role in the emergence of cholera epidemic in Iran (17). The present study showed that *V. cholerae* O1 was as the most important *V. cholerae* strain isolated from Iranian patients. Cholera is a self-limiting illness that is treatable with timely administration of fluids through oral route (4). However, in severely dehydrated patients, appropriate antibiotic therapy is needed (4). Reports have shown that the antibiotic resistance pattern of *V. cholerae* O1 has changed over the years and similar to global condition, spread of antibiotic resistance to commonly used antibiotics is increasing in Iran (13, 17). Therefore, due to increasing antimicrobial resistance, extensive antibiotic administration is not recommended (4). The most important

Table 2 - Antibiotic resistance patterns of *V. cholerae* in different provinces of Iran

Province	Antibiotic resistance (%) (95% CI)																	
	C	CIP	DOX	E	OXT	SXT	T	GM	F	S	PB	AP	AZ	NA	CFM	CP	K	AMX
Tehran	24.3 (4.3-69.4)	0	4.8 (1.7-12.8)	7.5 (0.3-69.1)	96 (69.6-99.6)	94.8 (43-99.8)	58.1 (2.5-98.7)	2.7 (0.7-10.3)	66.1 (0.1-100)	92.5 (19-99.8)	86.7 (59.5-96.6)	26.7 (10.4-53.3)	ND	ND	ND	ND	ND	ND
Alborz	ND	0	12.6 (1.4-59.8)	27.2 (1-93.5)	ND	87.2 (43.7-98.3)	15.6 (12-20.1)	ND	72.1 (37-91.9)	ND	ND	46 (39.8-52.4)	0.8 (0.2-3.3)	72 (60.8-81)	6.7 (2.8-15)	1.3 (0.2-8.9)	54.7 (43.4-65.5)	61.3 (49.9-71.6)
Khuzestan	7.7 (0.5-58.1)	0	4 (0.4-33)	11.8 (0.1-95.6)	95.8 (88.5-98.5)	97.1 (90.5-99.2)	79.6 (2.5-99.8)	4.3 (1.3-14)	33.3 (22.6-46.1)	99.2 (88.2-99.9)	ND	ND	ND	ND	ND	ND	ND	ND
Kerman	2.3 (0.3-14.4)	0	4.5 (1.1-16.4)	2.3 (0.3-14.4)	2.3 (0.3-14.4)	98.9 (84.6-99.9)	4.5 (1.1-16.4)	0	98.9 (84.6-99.9)	98.9 (84.6-99.9)	ND	ND	ND	ND	ND	ND	ND	ND
Kermanshah	33.9 (6.6-78.8)	0	0	36.4 (5.1-86)	64.2 (38.2-83.9)	60.6 (8.9-96.1)	0	0	90 (32.6-99.4)	90 (32.6-99.4)	ND	ND	ND	ND	ND	ND	ND	ND
Kashan	0	1.5 (0.5-4.3)	2.4 (1.1-5.5)	32.8 (1-95.8)	0	17.4 (10.5-27.4)	2.4 (1.1-5.5)	0	17.7 (4.9-47.3)	98.3 (78.3-99.9)	ND	34.5 (27.4-42.3)	ND	29.2 (14.6-49.8)	37.5 (28.4-47.6)	ND	ND	10.3 (4.7-21.2)
Sistan & Baluchastan	13.5 (0.7-78.3)	0	0	18.8 (2.1-71.1)	0	84.9 (64.2-94.6)	56.1 (36.1-74.3)	0	70.7 (40.1-89.7)	96.4 (86.6-99.1)	75 (37.7-93.7)	64.2 (15.5-94.6)	ND	90.4 (4.8-99.9)	ND	ND	ND	ND
Golestan	63.9 (17.2-93.8)	ND	15 (9.4-23.1)	32.4 (7.7-73.4)	15.8 (9.7-24.6)	70 (10.2-98)	41.9 (6.6-88)	0	20.2 (1-86.9)	92.9 (63-99)	71.4 (43.9-88.9)	45 (35.9-54.4)	ND	84.2 (75.4-90.3)	ND	ND	ND	ND
Qom	96.4 (61.6-99.8)	ND	0	0	ND	96.4 (61.6-99.8)	92.3 (60.9-98.9)	0	0	96.4 (61.6-99.8)	84.6 (54.9-96.1)	38.5 (17-65.6)	ND	ND	ND	ND	ND	ND
Hamadan	67 (57.2-75.5)	8.1 (4.8-13.5)	22.9 (9.7-45.2)	43.4 (15.4-76.4)	ND	68.6 (56.5-78.7)	23.3 (14.3-35.6)	ND	99.4 (95.6-99.9)	ND	ND	92 (84.8-95.9)	ND	2 (0.5-7.6)	ND	ND	11.7 (5.7-22.5)	ND
Qazvin	45.1 (32.1-58.8)	ND	49 (35.7-62.5)	62.7 (48.8-74.8)	ND	ND	43.1 (30.4-56.9)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Guilan	19 (13.4-26.3)	0.7 (0.1-4.8)	8.5 (4.9-14.3)	5.6 (2.8-10.9)	ND	72.5 (64.6-79.2)	16.2 (11-23.2)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	33.6 (21.9-47.8)	1.8 (1-3.1)	7.2 (4.3-11.6)	18.6 (11.3-28.9)	40.2 (16.9-68.8)	86 (77.6-91.7)	34.5 (23.7-47.3)	2.8 (1.5-5)	69.8 (50.7-83.9)	93.8 (88.7-96.7)	80.7 (70-88.3)	32.1 (20.7-46)	0.8 (0.2-3.3)	88.9 (70-96.5)	3.7 (0.6-20)	1.3 (0.2-8.9)	29 (4.5-78.1)	30.5 (3.3-85.1)

Abbreviations: C-chloramphenicol; CIP-ciprofloxacin; DOX-doxycycline; E-erythromycin; OXT-oxytetracycline; SXT-trimethoprim/sulphamethoxazole or co-trimoxazole; T-tetracycline; GM-gentamicin; F-furazolidone; S-streptomycin; PB-polymyxin; AP-ampicillin; AZ-azithromycin; NA-nalidixic acid; CFM-cefixime; CP-cefepime; K-kanamycin; AMX-amoxicillin; ND- Not determined.

antibiotics used to reduce the symptoms of cholera are cell growth inhibitors (penicillin, ampicillin, and vancomycin), protein synthesis inhibitors (erythromycin, chloramphenicol, streptomycin, kanamycin, gentamicin, spectinomycin, tetracycline, doxycycline and linezolid), folic acid metabolism inhibitors (trimethoprim and sulfamethoxazole), DNA replication inhibitors (ciprofloxacin, nalidixic acid and norfloxacin) and inducers of cell lysis/cytotoxicity (polymyxin B) (40). Tetracyclines and quinolones antibiotics were extensively used against *V. cholera* but resistance is relatively common and has restricted their application in severe cases (40). In these cases, co-administration of doxycycline along with oral rehydration is recommended (40). In the present study, resistance high rates of *V. cholerae* against tested tetracyclines and quinolones antibiotics including oxytetracycline (40.2%), tetracycline (34.5%), furazolidone (69.8%) and nalidixic acid (88.9%) were found in Iran. However, resistance rates to ciprofloxacin (1.8%) and doxycycline (7.2%) were low (Table 2). Dengo-Baloi *et al*. reported that *V. cholerae* O1 resistance to tetracycline, doxycycline, ciprofloxacin and nalidixic acid were 50%, 56%, 0% and 100%, respectively, in Mozambique (41). Chomvarin *et al*. reported antibiotic susceptibility pattern of *V. cholerae* O1 in Thailand as follows: 23% to tetracycline, and 0% to ciprofloxacin (42). Additionally, in Ghana, resistance profiles were 0% to tetracycline, 98.4% to ciprofloxacin and 100% to nalidixic acid (43). Erythromycin and furazolidone are used as alternatives to tetracyclines in children and pregnant women infected with cholera (44). However, according to the results of the current study, resistance to furazolidone is relatively common in Iran (69.8%) (Table 2). Therefore, in young children, antibiotic therapy with erythromycin is recommended (40). Additionally, due to low macrolide resistance of *V. cholera*, these antibiotics are the drugs of choice for treatment of children and adults, especially azithromycin (45). In this study, *V. cholerae* resistance rates to erythromycin and azithromycin were 18.6% and 0.8%, respectively (Figure 2). Resistance rate of *V. cholerae* strains to macrolides in Iran was higher than in Mozambique (13% for azithromycin) and lower than in Thailand (71% for erythromycin) (41, 42). Bacterial efflux pumps, spontaneous mutations, SXT elements and mobile integrons, and conjugative plasmids are the main mechanisms of antibiotic resistance in *V. cholerae* strains (40). Studies have suggested that the dominant antibiotic-resistance mechanisms of *V. cholera* against tetracycline and ciprofloxacin are VcaM (a bacterial ATP-driven efflux pump) and other types of *V. cholerae* efflux pumps belonging to the proton-motive force (PMF) pump family including MFS (major facilitator superfamily) that confers resistance to chloramphenicol and nalidixic acid, and RND (resistance–nodulation–cell division) systems that confers resistance to polymyxin B and erythromycin (40). However, the studies included in this meta-analysis did not evaluate antibiotic-resistance mechanisms of *V. cholera*. This meta-analysis showed that *V. cholerae* resistance rates to β-lactam antibiotics and aminoglycosides were variable and a higher resistance rate to ampicillin (32.1%), amoxicillin (30.5%), streptomycin (93.8%) and kanamycin (29%) was found compared to other β-lactam antibiotics and aminoglycosides such as cefixime (3.7%), cefepime (1.3%) and gentamicin (2.8%). Resistance to ampicillin was reported to be 100%, 31% and 95.2% in Mozambique, Thailand and Ghana, respectively (41-43), whereas sensitivity to gentamicin was reported in Thailand and Ghana (42, 43). Chloramphenicol is also another protein synthesis inhibitor that is commonly used to treat cholera infection. Our study showed that *V. cholerae* resistance

to chloramphenicol was relatively high in Iran (33.6%). Mechanisms of antibiotic resistance were not evaluated in the included studies. However, based on other studies, spontaneous chromosomal mutations may be involved (40). Resistance to chloramphenicol in Iran was higher than in Ghana (0%) and lower than in Mozambique (89%) (41, 43). Finally, *V. cholerae* resistance to trimethoprim/sulphamethoxazole was also high in Iran (86%). The results were consistent with reports from Mozambique (75%), Thailand (54%) and Ghana (96.8%) (41-43). Among the included studies, Adabi *et al*. (13) showed that SXT elements and mobile integrons, which harbor resistance genes to trimethoprim/sulphamethoxazole, chloramphenicol and streptomycin, are frequently present in clinical *V. cholerae* O1 isolates of Iran. However, further investigations are needed to determine the exact mechanisms of resistance.

Conclusions

The present meta-analysis on the epidemiology of antibiotic resistance showed that the *V. cholerae* O1 strains isolated in Iran exhibited a high resistance rate against numerous antibiotics, including chloramphenicol, oxytetracycline, trimethoprim/sulphamethoxazole, tetracycline, furazolidone, streptomycin, polymyxin B, ampicillin, nalidixic acid, kanamycin and amoxicillin. Therefore, antibiotic therapy with ciprofloxacin, doxycycline, erythromycin, gentamicin, azithromycin, cefixime and cefepime could be more effective for severe cases of cholera in Iran. Exploring different mechanisms of antibiotic resistance and the dominant antibiotic-resistant elements as well as continuous monitoring of *V. cholerae* antibiotic resistance seems to be necessary to achieve a better control of cholera in Iran.

Riassunto

Revisione sistematica con metanalisi dell'epidemiologia dell'antibioticoresistenza di** Vibrio cholerae **in Iran

Premessa. Il colera, una malattia diarroica acuta infettiva provocata dal *Vibrio cholerae*, è endemico in Iran, dove rappresenta un importante problema di Sanità Pubblica. La terapia antibiotica è in grado di ridurre la durata della malattia, la trasmissione dell'infezione e la contaminazione dell'ambiente. Tenendo presenti i diversi profili di resistenza antibiotica del *V. cholerae* osservati a livello globale, lo scopo della presente revisione sistematica con metanalisi è stato quello di valutare la prevalenza delle antibioticoresistenze del *V. cholerae* in Iran.

Metodi. È stata effettuata una revisione sistematica della letteratura, usando parole chiave, nelle basi dati elettroniche nazionali (Irandoc, Iran Medex, Magiran) ed internazionali (PubMed, Scopus, Google Scholar ed ISI Web of Knowledge). A tutto il 31 Luglio 2018, sulla base di ben definiti criteri di inclusione ed esclusione, sono stati recuperati per la metanalisi 27 lavori.

Risultati. Il *V. cholerae* O1 è risultato il ceppo prevalente tra quelli isolati in Iran, ed ha esibito un elevato tasso di resistenza verso numerosi antibiotici, tra cui il cloramfenicolo (33,6%), l'ossitetraciclina (40,2%), il trimetoprim/sulfametossazolo (86%), la tetraciclina (34,5%), il furazolidone (69,8%), la streptomicina (93,8%), la polimixina (80,7%), l'ampicillina (32,1%), l'acido nalidixico (88,9%), la kanamicina (29%) e l'amoxicillina (30,5%).

Conclusioni. Sulla base dei risultati della metanalisi, l'antibioticoterapia con ciprofloxacina, doxiciclina, eritromicina, gentamicina, azitromicina, cefixime e cefepime può risultare efficace per il trattamento dei casi gravi di colera in Iran.

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