Global status of tetracycline resistance among clinical isolates of *Vibrio cholerae*: a systematic review and meta-analysis

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**Abstract**

**Background:** There has been an increasing resistance rate to tetracyclines, the first line treatment for cholera disease caused by *V. cholera* strains, worldwide. The aim of the present study was to determine the global status of resistance to this class of antibiotic among *V. cholera* isolates.

**Methods:** For the study, electronic databases were searched using the appropriate keywords including: 'Vibrio,' 'cholera,' 'Vibrio cholerae', 'V. cholerae', 'resistance,' 'antibiotic resistance,' 'antibiotic susceptibility,' 'antimicrobial resistance,' 'antimicrobial susceptibility,' 'tetracycline,' and 'doxycycline.' Finally, after some exclusion, 52 studies from different countries were selected and included in the study and meta-analysis was performed on the collected data.

**Results:** The average resistance rate for serogroup O1 to tetracycline and doxycycline was 50% and 28%, respectively (95% CI). A high level of heterogeneity ($I^2 > 50\%$, $p$-value < 0.05) was observed in the studies representing resistance to tetracycline and doxycycline in O1 and non-O1, non-O139 serogroups. The Begg's tests did not indicate the publication bias ($p$-value > 0.05). However, the Egger's tests showed some evidence of publication bias in the studies conducted on serogroup O1.

**Conclusions:** The results of the present study show that the overall resistance to tetracyclines is relatively high and prevalent among *V. cholerae* isolates, throughout the world. This highlights the necessity of performing standard antimicrobial susceptibility testing prior to treatment choice along with monitoring and management of antibiotic resistance patterns of *V. cholerae* strains in order to reduce the emergence and propagation of antibiotic resistant strains as well as the failure of treatment.

**Keywords:** *Vibrio cholerae*, Tetracycline, Doxycycline, Resistance

**Introduction**

Cholera is an ancient infectious disease mainly affecting developing countries. The disease is capable to spread across many countries leading to vast pandemics and becoming a major public health concern throughout the world [1].

The causative agent of this life threatening diarrheal disease is *Vibrio cholerae* secreting the cholera toxin. Two major cholera toxin-producing serogroups of this bacterial pathogen, O1 and O139, have potential to spread and cause epidemic as well as pandemic disease [2]. The serogroup O1 has two biotypes, classical and El Tor, and each biotype has three serotypes including Ogawa, Inaba, and Hikojima [1, 3].

The main stay of management of cholera (acute gastroenteritis) is urgent fluid replacement; however, the use of an appropriate antibiotic is necessary to eliminate the
bacteria, lessen the duration of illness, and control the disease [4].

Tetracyclines (tetracycline and doxycycline) have long been the antibiotics of choice for treating severe cholera effectively worldwide, except for young children and pregnant women [5]. However, tetracycline resistant strains of *V. cholerae* are being increasingly reported worldwide. These resistant strains have been responsible for major epidemics in some countries and geographical areas such as Latin America, Tanzania, Bangladesh, and Zaire [6].

To date, numerous studies have reported the different antibiotic resistance patterns for *V. cholerae* isolates throughout the world. Nevertheless, the overall status of the resistance to tetracyclines among the strains is not intensively studied.

The present study was conducted to determine the global status of resistance to the antibiotics of tetracycline family, including tetracycline and doxycycline, among different *V. cholerae* isolates using a systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [7].

**Methods**

**Search strategies**

The electronic databases, including OVID databases, PubMed, Web of Science, Scopus, MEDLINE, EMBASE, Cochrane Library, as well as Google Scholar, were searched for papers reporting the resistance rate for different *Vibrio cholerae* isolates to the antibiotics of tetracyclines family from December 1980 to April 2020. The search was restricted to original research articles throughout the world, published in English using the following keywords with the help of Boolean operators (AND, OR): ‘*Vibrio*, *cholera*, *Vibrio cholerae*, ‘*V. cholerae*, ‘resistance’, ‘antibiotic resistance’, ‘antibiotic susceptibility’, ‘antimicrobial resistance’, ‘antimicrobial susceptibility’, ‘tetracycline’, and ‘doxycycline’. References from reviewed articles were also searched for more information.

**Inclusion and exclusion criteria**

Included studies were all original research articles as well as some letter to editors presenting the resistance rates for *Vibrio cholerae* isolates to the tetracyclines including tetracycline and doxycycline.

Excluded articles were those that: (1) had no sufficient data to be analyzed; (2) reported antibiotic resistance of *Vibrio* species other than *V. cholerae*; (3) studied resistance to antibiotics other than tetracyclines; and (4) tested environmental isolates of the bacterium instead of clinical ones; for example, the strains isolated from wastewater, water supplies, river, aquaculture water, fishery products, as well as seafood.

Review articles, congress abstracts, studies reported in languages other than English, meta-analyses or systematic reviews, duplicate publications of the same study and articles available only in abstract form were also excluded.

**Data extraction**

The data extracted from each study included first author’s name, year of publication, geographical area of study (country), clinical sample (specimen type), serogroup, biotype, and serotype of the isolates, number of investigated isolates (sample size), method of susceptibility testing, and number of isolates resistant to each antibiotic.

**Statistical analysis**

The data were analyzed using Comprehensive Meta-Analysis Software Version 2.0 (Biostat, Englewood, NJ, USA). The resistance rate was reported by 95% confidence intervals (CIs).

Cochrane Q-statistic test and *I*² test were performed to estimate heterogeneity between studies, and in all calculations of which *I*² was above 50%, the random effect model was chosen to estimate the average rate because of its conservative summary estimate; otherwise, the fixed effect model was applied. To assess possible publication bias, a funnel plot, Begg’s rank correlation and Egger’s weighted regression methods were used. Two-tailed *p* < 0.05 was considered indicative of a significant publication bias. The relative weight for each study was also calculated.

**Results**

A total of 138 articles were collected for assessment. Through the first screening, 15 articles were excluded on the basis of the title evaluation, as nine of them were duplicate publications of the same study, and six have titles irrelevant to the present study. By the second assessment, nine papers were discarded because they had represented the study of *Vibrio* species other than *V. cholerae*, or were review articles. Finally, after full-text evaluation, 62 studies were ruled out because they had reported resistance to antibiotics other than tetracyclines, used environmental isolates of the bacterium instead of clinical ones, and/or had no sufficient data. Therefore, 52 articles published between 1980–2020 were selected and included in the final analysis (Fig. 1 and Table 1).

The included studies were carried out in 20 different countries, majority of which (38 studies) located in Asia, 12 in Europe, one in Caribbean, and one in Oceania (Table 1).
Fig. 1 Flow chart of the literature search, systematic review and study selection. *Articles representing study of *Vibrio* species other than *V. cholerae*; **Articles reported resistance to antibiotics other than tetracyclines; ***Studies using environmental instead of clinical isolates of *V. cholerae*. 

English databases: OVID databases, PubMed, Scopus, Web of Science, MEDLINE, EMBASE, Cochrane Library, and Google Scholar.

N = 138

Excluded:
Duplicates: n = 9
Title not relevant: n = 6

Abstract evaluation:
N = 123

Excluded:
Other *Vibrio* spp.: n = 5
Review articles: n = 4

Full-text evaluation:
N = 114

Excluded:
Antibiotics other than tetracyclines**: n = 22
Environmental (non-clinical) isolates**: n = 31
No sufficient data: n = 9

Articles included in meta-analysis:
N = 52
| References          | Pub. year | Country | Source         | Serogroup/biotype | Serotype | No. of isolates | No. of resistant isolate (%) | Method of susceptibility testing |
|---------------------|-----------|---------|----------------|-------------------|----------|----------------|-----------------------------|--------------------------------|
| Olipher et al.      | 2020      | Kenya   | Stool          | Non-O1            | ND       | 98             | 64 (65.3) NM                | Kirby–Bauer disk diffusion     |
| Kale et al.         | 2020      | India   | Stool          | O1/El Tor         | Ogawa    | 109            | 0 (0) 0 (0)                 | Kirby–Bauer disk diffusion     |
| Abana et al.        | 2019      | Ghana   | ND             | O1/El Tor         | Ogawa    | 40             | 14 (35) 6 (15)              | Kirby–Bauer disk diffusion     |
| Zereen et al.       | 2019      | Bangladesh | Stool   | ND/ND            | ND       | 3              | 2 (66.7) NM                | Kirby–Bauer disk diffusion     |
| Sreedhara and Mohan | 2019      | India   | Stool          | O1/El Tor         | Ogawa    | 74 TET, 41 DOX | 19 (25.7) 10 (24.4)         | Kirby–Bauer disk diffusion     |
| Dua et al.          | 2018      | India   | Stool          | Non-O1, non-O139  | Ogawa    | 71             | 29 (40.9) 63 (88.7)         | Kirby–Bauer disk diffusion     |
| Uddin et al.        | 2018      | Bangladesh | Stool    | O1/ND            | 43 Ogawa & 15 Inaba | 58              | 17 (29.3) 10 (17.2)         | Kirby–Bauer disk diffusion     |
| Fernández-Abreu et al. | 2017   | Cuba    | Stool          | Non-O1, non-O139  | ND       | 125            | 5 (4) 1 (0.8)               | Kirby–Bauer disk diffusion     |
| Shah et al.         | 2017      | Pakistan | Stool & vomitus | ND/ND            | ND       | 131            | 13 (9.9) NM                | Kirby–Bauer disk diffusion     |
| Dengo-Baloi et al.  | 2017      | Mozambique | Rectal swabs | O1/El Tor         | Ogawa    | 159            | 79 (50) 89 (56)            | Kirby–Bauer disk diffusion     |
| Patil et al.        | 2017      | India   | Stool          | O1/El Tor         | Ogawa    | 106            | 10 (9.4) NM                | Kirby–Bauer disk diffusion     |
| Jain et al.         | 2016      | India   | Rectal swabs   | O1/El Tor         | Ogawa    | 27             | 27 (100) NM                | Kirby–Bauer disk diffusion     |
| Hajia et al.        | 2016      | Iran    | ND             | O1/ND             | Ogawa & Inaba | 192            | 115 (59.9) NM              | Liofilchem Test Strip          |
| Torane et al.       | 2016      | India   | Stool          | O1/El Tor         | 407 Ogawa & 32 Inaba | 439            | 55 (12.5) NM              | Kirby–Bauer disk diffusion     |
| Gupta et al.        | 2016      | Nepal   | Stool          | O1/El Tor         | Ogawa    | 31             | 0 (0) 0 (0)                 | Agar dilution                  |
| Masoumi-Asl et al.  | 2016      | Iran    | Stool          | O1/ND             | Inaba    | 60             | 60 (100) NM                | Liofilchem Test Strip          |
| Irfan et al.        | 2016      | Pakistan | Stool         | Non-O1, non-O139  | ND       | 233            | 5 (2.1) NM                 | Kirby–Bauer disk diffusion     |
| Afzali et al.       | 2016      | Iran    | Stool          | Non-O1, non-O139  | ND       | 96             | 3 (3.1) 3 (3.1)            | Kirby–Bauer disk diffusion     |
| Kar et al.          | 2015      | India   | Rectal swabs   | O1/El Tor         | Ogawa    | 35             | 35 (100) NM                | Kirby–Bauer disk diffusion     |
| Ukaji et al.        | 2015      | Nigeria | Stool          | O1/ND             | ND       | 63             | 53 (84.1) NM               | Kirby–Bauer disk diffusion     |
| Tabatabaei and Khorasdad | 2015 | Iran    | Stool          | O1/ND             | Inaba    | 48             | 29 (60.4) NM               | Kirby–Bauer disk diffusion     |
| Barati et al.       | 2015      | Iran    | Rectal swabs   | O1/El Tor         | Ogawa    | 239            | 36 (15.1) 10 (4.2)         | Kirby–Bauer disk diffusion     |
| Mishra et al.       | 2015      | India   | Stool          | O1/El Tor         | Ogawa    | 44             | 0 (0) NM                   | Kirby–Bauer disk diffusion     |
| Kuma et al.         | 2014      | Ghana   | Stool & vomitus | O1/ND            | ND       | 275            | 43 (15.6) 40 (14.5)        | Kirby–Bauer disk diffusion     |
| Mercy et al.        | 2014      | Kenya   | ND             | O1/El Tor         | Inaba (most common) & Ogawa | 44              | 0 (0) 0 (0)                 | Kirby–Bauer disk diffusion     |
Table 1 (continued)

| References         | Pub. year | Country       | Source                     | Serogroup/| Serotype | No. of isolates | No. of resistant isolate (%) | Method of susceptibility testing |
|--------------------|-----------|---------------|----------------------------| biotype   |          |                |                            |                                 |
| Mahmud et al. [31] | 2014      | Sierra Leone  | Rectal swabs               | O1/El Tor | Ogawa    | 15             | 0 (0)                       | Kirby–Bauer disk diffusion & E-test |
| Murhekar et al. [32]| 2013      | Papua New Guinea | Stool & rectal swabs       | O1/El Tor | Ogawa    | 299            | 29 (9.7)                    | Kirby–Bauer disk diffusion |
| Tran et al. [33]   | 2012      | Vietnam        | ND                         | O1/El Tor | Ogawa    | 100            | 29 (29)                     | E-test strips                  |
| Sang et al. [34]   | 2012      | Kenya          | Stool                      | O1/ND     |          | 4              | 0 (0)                       | Kirby–Bauer disk diffusion |
| Mandal et al. [35] | 2012      | India          | Stool                      | O1/El Tor | Ogawa & 4 Inaba | 154      | 26 (16.9)                   | Agar dilution & E-test          |
| Shujatullah et al. [36] | 2012 | India          | Stool                      | O1/ND     | Ogawa (most common) & Inaba | 66       | 9 (13.6)                   | Kirby–Bauer disk diffusion |
| Borkakoty et al. [37] | 2012 | India          | Rectal swabs               | O1/El Tor | 24 Ogawa & 16 Inaba | 40       | 16 (40)                   | Kirby–Bauer disk diffusion |
| Das et al. [38]    | 2011      | India          | Stool & rectal swabs       | O1/ND     | Ogawa (most common), Inaba, Hikojima | 238      | 41 (17.2)                  | Kirby–Bauer disk diffusion & broth dilution |
| Karki et al. [39]  | 2011      | Nepal          | Stool                      | O1/El Tor | Ogawa    | 57             | 0 (0)                       | Kirby–Bauer disk diffusion |
| Rahbar et al. [40] | 2010      | Iran           | Stool & rectal swabs       | O1/El Tor | 199 Inaba & 21 Ogawa | 220      | 0 (0)                     | Kirby–Bauer disk diffusion |
| Abera et al. [41]  | 2010      | Ethiopia       | Stool                      | O1/ND     | Inaba     | 81             | 5 (6.2)                     | Kirby–Bauer disk diffusion |
| Supawat et al. [42] | 2009 | Thailand       | Stool & rectal swabs, blood | O1/ND     | 1032 Inaba & 43 Ogawa | 1075     | 16 (1.5)                  | Kirby–Bauer disk diffusion |
| Keramat et al. [43] | 2008      | Iran           | Stool                      | O1/El Tor | Inaba     | 60             | 14 (23.3)                   | Kirby–Bauer disk diffusion |
| Roychowdhury et al. [44] | 2008 | India          | Stool & rectal swabs       | O1/ND     | Inaba & Ogawa | 51       | 9 (17.6)                  | Kirby–Bauer disk diffusion |
| Mandomando et al. [45] | 2007 | Mozambique     | Rectal swabs               | O1/ND     | Ogawa    | 75             | 73 (97.3)                   | Kirby–Bauer disk diffusion |
| Faruque et al. [46] | 2007      | Bangladesh     | ND                         | O1/ND     | 762 Ogawa & 535 Inaba | 1297     | 711 (54.8)                | Kirby–Bauer disk diffusion |
| Rafi et al. [46]   | 2004      | Pakistan       | Stool                      | O1/66 El Tor & 57 Classical ND | 123     | 37 (30.1)                | Kirby–Bauer disk diffusion |
| Tjaniadi et al. [47] | 2003     | Indonesia      | Stool & rectal swabs       | O1/ND     | 1044 Inaba & 57 Classical ND | 68       | 12 (1.2)                  | Kirby–Bauer disk diffusion |
| Dromigny et al. [48] | 2002      | Madagascar     | Stool                      | O1/El Tor | ND       | 351            | 55 (15.7)                   | Kirby–Bauer disk diffusion |
| Sabeena et al. [49] | 2001      | India          | Stool                      | O1/El Tor | Ogawa    | 25             | 2 (8.0)                     | Kirby–Bauer disk diffusion |
| Ivanaga et al. [50] | 2000      | Laos           | ND                         | O1/El Tor | Ogawa    | 99             | 95 (95.9)                   | Agar dilution                  |
| Urassa et al. [51] | 2000      | Tanzania       | Stool                      | O1/ND     | ND       | 181            | 42 (23.2)                   | Kirby–Bauer disk diffusion |
| Garg et al. [52]   | 2000      | India          | ND                         | O1/ND     | Ogawa & ND | 326         | 8 (2.5)                     | Kirby–Bauer disk diffusion |

TET DOX
Of 52 articles included, 40 had studied only O1 serogroup all of which reported El Tor biotype, five had detected only non-O1, non-O139 serogroup, four had investigated O1, O139, and/or non-O1, non-O139 serogroups simultaneously, and three did not determined the serogroups of isolated *V. cholerae*. From the included studies, only one had detected and tested classical biotype beside the El Tor one.

The most commonly collected samples for assessment in the included studies were stool, and rectal swabs, but other samples included vomitus and blood (for isolation of non O1, non O139 *V. cholerae*).

The included studies tested antimicrobial susceptibility to tetracycline and doxycycline for the serogroups O1 (44 and 16 studies, respectively), O139 (two and one studies, respectively), and non-O1, non-O139 (eight and three studies, respectively) of *V. cholerae*. From the studies conducted on serogroup O1, 19 detected only Ogawa, three only Inaba, and 12 detected both serotypes, simultaneously. Only one study detected Hikojima serotype along with other two serotypes, concurrently. The remaining studies conducted on serogroup O1 did not determine the serotypes of their isolates.

The studies mainly used Kirby-Bauer disk diffusion method for susceptibility testing, but other techniques were broth and agar dilution, E-test, and Liofilchem Test Strip.

The number of *V. cholerae* isolates investigated (sample sizes) in the studies varied from 3–1297. The range of antibiotic resistance as well as the pooled resistance rate for *V. cholerae* isolates (serogroups O1, O139, and non-O1, non-O139) to tetracycline and doxycycline are shown in Table 2.

The average resistance rate for serogroup O1 to tetracycline and doxycycline was 50% and 28%, respectively (95% CI). Figures 2a–c and 3a–c show the forest plots of the meta-analysis for resistance rate of different serogroups of *V. cholerae* to the antibiotics. A high level of heterogeneity (*I² > 50%, p-value < 0.05*) was observed in the studies representing resistance to tetracycline and doxycycline in O1 and non-O1, non-O139 serogroups; however, the number of included studies conducted on the antimicrobial resistance of O139, as well as non-O1, non-O139 serogroups to tetracycline and doxycycline was fewer than 10 and insufficient for an accurate analysis.
Fig. 2  Forest plots of the meta-analysis for resistance rate of V. cholerae serogroups O1 (a), O139 (b), and Non-O1, non-O139 (c) to tetracycline.
Fig. 3 Forest plots of the meta-analysis for resistance rate of *V. cholerae* serogroups O1 (a), O139 (b), and Non-O1, non-O139 (c) to doxycycline.
The Begg’s tests did not indicate the publication bias ($p$-value > 0.05). However, the Egger’s tests showed some evidence of publication bias in the studies conducted on serogroup O1 (Table 2). The corresponding funnel plots of the all the analyses (except serogroup O139 in which the number of included studies was fewer than three and insufficient for application of funnel plot), are shown in Fig. 4a–d.

**Discussion**

The historical disease, cholera, has been endemic in south Asia, especially the Ganges delta region in Bangladesh and India, from which the disease spread outside the Indian subcontinent along trade routes causing the pandemics with high mortality rates (millions of deaths) throughout the world [2]. To date, toxigenic *Vibrio cholerae* (O1 serogroup) has caused seven pandemics, six of which were due to classical biotype and the seventh pandemic caused by El Tor one [37].

Although the antibiotics cannot be used as a sole treatment for the disease; however, combining fluid replacement therapy with antibiotic treatment has advantages as the antibiotics could lessen the duration of illness and reduce shedding of *V. cholerae* in the stool [4].

Tetracyclines are ‘broad-spectrum antibiotics’ that inhibit the bacterial 30S ribosomal subunit and consequent protein synthesis [57]. These antibiotics, particularly tetracycline and doxycycline, have long been the antibiotics of choice for treating severe cholera around the world, except for young children and pregnant women [2, 5]. However, tetracycline-resistant strains of *V. cholerae* have been emerged continuously over the years, due mainly to the extensive clinical and non-clinical uses of this class of antibiotic [6, 52].

By performing this systematic review and meta-analysis, it was found that the resistance rate of *V. cholerae* isolates to tetracyclines was greatly variable in various studies conducted in different geographical areas. The regional differences in resistance rate of *V. cholerae* isolates to tetracyclines may result from various exposure of patients in different populations to the antibiotics. This highlights the necessity of regional and local antibiotic susceptibility testing before antibiotic administration to avoid failure of treatment.

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**Fig. 4** Funnel plots of the meta-analysis for resistance rate of *V. cholerae* serogroups O1 and Non-O1, non-O139 to tetracycline (a, b) and doxycycline (c, d), respectively. (In case of serogroup O139, the number of included studies was fewer than three and insufficient for application of funnel plot)
The high level of heterogeneity in the studies as well as the differences in sample sizes might impact on the analyses. To overcome this problem, the relative weight for each study was calculated and considered in the present study. Another problem in the current study was that the number of included studies conducted on the antimicrobial resistance of O139 and non-O1, non-O139 serogroups of \textit{V. cholerae} was fewer than 10 and insufficient for a powerful meta-analysis and an accurate conclusion.

The results of the present meta-analysis showed that the overall resistance rate of \textit{V. cholerae} isolates to tetracyclines (including tetracycline and doxycycline) was relatively high and between these two antibiotics, the average resistance rate to tetracycline was higher in serogroup O1.

Tetracycline resistance in \textit{V. cholerae} isolates has been reported from Bangladesh since 1979 [56]. As with other antibiotics, the genes encoding resistance to tetracyclines commonly locate on mobile genetic elements such as plasmids and transposons by which the genes could be rapidly transferred and exchanged among the clinical as well as environmental strains of \textit{V. cholera}, leading to increased resistance to these antibiotics [58]. Moreover, the antibiotic resistance determinants may be transferred and exchanged between environmental and clinical isolates of \textit{V. cholerae} through the horizontal gene transfer mechanisms [59]. These events lead to rapid increase in antibiotic resistance among the isolates.

Among the involved mechanisms of resistance to tetracyclines, the active efflux of antibiotic from bacterial cell as well as the production of ribosomal protection proteins (encoded by \textit{tet} genes) are predominant in clinical settings. The other implicated mechanisms are target site mutation, decreased drug permeability, and enzymatic degradation of the antibiotic [58].

It has been evidenced that classical biotype of \textit{V. cholerae} generally causes more severe illness compared to El Tor counterpart; in turn, the latter biotype is more adaptable and flexible in the environment, has more asymptomatic carriers, and causes higher infection to case ratio [37]. Furthermore, it has been shown that the strains of \textit{Vibrio cholerae} serogroup O1 may change the biotype from Ogawa to Inaba and vice versa. Such biotype interconversion has been linked to variation in antibiotic resistance in some cases [18].

Besides tetracyclines as the first line drugs, the other antibiotic options for treatment of severe cholera include furazolidone, ciprofloxacin, erythromycin, trimethoprim-sulphamethoxazole, and chloramphenicol [5]. However, the emergence of multiple antibiotic resistant strains of \textit{V. cholerae} (displaying resistance against several antibiotics) is a major global issue and a serious problem for public health. The reasons for the appearance and development of such resistant strains may be attributed to the extensive misuse of antibiotics without proper susceptibility testing as well as the lack of an appropriate national surveillance program to monitor the bacterial resistance patterns [6]. For example, the emergence of tetracycline resistant strains causing an epidemic in Tanzania was due to the widespread use of this antibiotic for prophylaxis [50].

On the other hand, in some countries, wastewater and human excreta are routinely used for farming or in the aquaculture systems. This causes the shedding of \textit{Vibrio cholerae} to these environments. It is known that antibiotics are also disseminated into the environment in many ways such as excretion from humans or animals (through urine and feces), farming, and/or disposal of antimicrobials. The degradation of some antibiotics including tetracyclines takes a considerably longer time. Therefore, these antibiotics remain in water for a long period of time and gradually accumulate to reach a higher concentration. Consequently, the exposure of \textit{V. cholerae} strains to these antibiotics in environmental settings, may lead to development and increase of resistant strains in aquatic ecosystem through natural selection. Eventually, the aquatic ecosystem as well as aquatic products serve as important reservoirs for antibiotic resistant as well as more virulent \textit{Vibrio cholerae} strains capable to spread and transmit to humans via direct contact or through the food chain [59], thereby causing the epidemic infections characterized by failure in treatment.

The antimicrobial susceptibility testing according to approved CLSI guidelines (M45) is necessary prior to treatment choice [60]. However, it has been revealed that in vitro susceptibility of \textit{V. cholerae} to antibiotics does not necessarily correlate with in vivo activity [3]. Recently, some non-antibiotic techniques have been introduced as possible alternatives to traditional antibiotics in order to control pathogens and minimize the risk of development of antibiotic-resistant strains in the environment. These possible alternatives may include inhibition of bacterial quorum sensing (quorum quenching), application of bacteriophages, and using of probiotics [59]. Moreover, it is notable that some vaccines are currently licensed or under development for prophylaxis against cholera disease in children and adults as reviewed by Shaikh et al. [61].

**Conclusion**

In conclusion, the results of the present study show that the overall resistance to tetracyclines, the first line treatment for cholera disease, is relatively high and prevalent among \textit{V. cholerae} isolates, throughout the world. Hence, performing regional antimicrobial susceptibility testing according to approved CLSI guidelines prior to treatment...
choice along with monitoring and management of antibiotic resistance patterns of *V. cholerae* strains seems to be necessary. In this regard, planning the national or international surveillance programs would be helpful to reduce the emergence and propagation of antibiotic resistant strains as well as the failure of treatment.

**Abbreviations**

CI: Confidence interval; PRISMA: Preferred reporting items for systematic reviews and meta-analyses.

**Acknowledgements**

None.

**Authors’ contributions**

MHA is the only author of the article. The author read and approved the final manuscript.

**Funding**

None.

**Availability of data and materials**

All data generated by this study have been submitted with this manuscript.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The author declares that he has no competing interests.

**Received:** 24 March 2021   **Accepted:** 19 July 2021

**Published online:** 06 August 2021

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