REVIEW

The prevalence of antibiotic-resistant *Clostridium* species in Iran: a meta-analysis

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ABSTRACT

Clostridium species are ubiquitous and associated with various diseases in animals and humans. However, there is little knowledge about the prevalence of their resistance to antibiotics in Iran. Therefore, the aim of this study was to determine the prevalence of antibiotic-resistant *Clostridium* species in Iran through a meta-analysis of eligible studies published up until December 2018. Fourteen articles on the drug resistance of *Clostridium* species in Iran were included in the current study following a search in PubMed, Scopus and Google Scholar databases using relevant keywords and screening based on inclusion and exclusion criteria. Antibiotic resistance rates of *C. difficile* to ampicillin (42.8%), ciprofloxacin (69.5%), clindamycin (84.3%), erythromycin (61.5%), gentamicin (93.5%), nalidixic acid (92.9%), tetracycline (32.5%), imipenem (39.6%), levofloxacin (93.4%), ertapenem (58.7%), piperacillin/tazobactam (56.5%), kanamycin (100%), colistin (100%), ceftazidime (76%), amikacin (76.5%), moxifloxacin (67.9%) and cefotaxime (95%) were high. In addition, resistance of *C. perfringens* to ampicillin (25.8%), erythromycin (32.9%), gentamicin (45.4%), nalidixic acid (52.5%), tetracycline (19.5%), penicillin (21.8%), trimethoprim-sulfamethoxazole (32.1%), amoxicillin (19.3%), imipenem (38%), cloxacillin (100%), oxacillin (45.6%), bacitracin (89.1%) and colistin (40%) was high. Metronidazole and vancomycin, as the first-line therapies, fidaxomicin, tetracyclines (except tetracycline), rifampicin and chloramphenicol can still be used for the treatment of *C. difficile* infections. However, the present results do not recommend the use of penicillin, bacitracin and tetracycline for the treatment of *C. perfringens* infections in humans and domestic animals in Iran.

KEYWORDS

Drug resistance; *Clostridium*; Iran; meta-analysis

1. Introduction

A collection of Gram-positive and spore-forming anaerobic rods are classified in the genus *Clostridium*. Among the species of this genus, *Clostridium difficile* (*C. difficile*), *Clostridium perfringens* (*C. perfringens*), *Clostridium tetani* (*C. tetani*) and *Clostridium botulinum* (*C. botulinum*) are ubiquitous and the most common pathogens associated with human and animal diseases [1]. *C. difficile* colonizes in the human colon and is responsible for hospital-associated diarrhea and two antibiotic-related infections including antibiotic-associated diarrhea and pseudo-membranous colitis [2,3]. *C. difficile* infections (CDI) can occur in those receiving antibiotics and as a healthcare-associated infection in hospitalized patients [4]. On the other hand, there are concerns about emerging community-associated diseases, increasing deaths and relapse rates associated with hypervirulent strains of *C. difficile*, especially ribotypes 027 (toxintype III) and 078 (toxintype V) [3]. *C. difficile*-associated diarrhea is more prevalent in children less than 5 years old, particularly those receiving long-term antibiotic therapy [5]. The main antibiotics that are associated with CDI are clindamycin,

expanded- and extended-cephalosporins and fluoroquinolones [6]. Antibiotic therapy with these drugs leads to the elimination of intestinal microbiota followed by replacement of resident or ingested *C. difficile* that can produce CDI by two toxins including toxin A (enterotoxin) and toxin B (cytotoxin) [6]. The Centers for Diseases Control and Prevention (CDC) has classified *C. difficile* together with carbapenem-resistant Enterobacteriaceae and drug-resistant *Neisseria gonorrhoeae* as an urgent medical threat level in the United States [4]. This bacterium causes around 250,000 infections, 14,000 deaths and \$1 billion medical costs per year in the United States [4]. According to CDC reports, because of the appropriate responses of *C. difficile* to prescribed antibiotics, the emergence of antibiotic resistance is not yet considerable [4]. However, *C. difficile* has inherent resistance to antibiotics used to treat other infections, which leads to rapid spread of CDI [4]. On the other hand, antibiotic resistance of *C. difficile* is associated with the emergence of new strain types as well as the occurrence/recurrence of CDI [6]. Additionally, there is no comprehensive information about the emergence of antibiotic-resistant *C. difficile* in other countries such as Iran. *C. perfringens* is another

obligate anaerobic Gram-positive organism which is localized in the intestinal tract of humans, animals and insects and also found in the environment such as in soil and water [7]. The bacterium can contaminate wounds and cause soft tissue infections including cellulitis, suppurative myositis and myonecrosis [1,7]. Enterotoxin-producing type A *C. perfringens* is responsible for gastroenteritis, including food poisoning and necrotizing enteritis after consuming meat products particularly raw meat and poultry [1,7]. The bacterium is also associated with serious and economically important diseases in animals such as necrotic enteritis in chickens [7]. Clostridial food poisoning can be treated with oral rehydration but soft tissue infections need surgical debridement and antibiotic therapy [1]. *C. tetani* and *C. botulinum* are ubiquitous microorganisms in soil and can lead to tetanus and botulism diseases through the contamination of wounds and the consumption of foods contaminated with bacterial spores [1]. Surgical debridement, antibiotic therapy and administration of antitoxin are the most commonly used methods in the treatment of tetanus and botulism [1].

Antibiotic resistance among anaerobic bacteria such as clostridial species is increasing worldwide. Different profiles of resistance in different regions may be due to selective antibiotic pressures, different methods used to determine antibiotic resistance patterns and lack of uniformity in the interpretation of results [8]. Generally, treatment for drug-resistant infections, especially multidrug resistant (MDR) strains, is costly and sometimes unsuccessful. Such infections cause doubling of the duration of hospital stay and significant rates of morbidity and mortality in both hospitals and communities [9]. There is limited information on the antibiotic resistance prevalence of *Clostridium* species in Iran. Therefore, the aim of this study was to determine the prevalence of antibiotic-resistant *Clostridium* species isolated from different specimens in Iran.

2. Methods

In the current systematic review and meta-analysis, literature search, selection criteria, data extraction and statistical analysis were performed according to the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [10].

2.1. Literature search

A systematic search was conducted in three electronic databases including PubMed (<https://www.ncbi.nlm.nih.gov>), Scopus (<https://www.scopus.com>) and Google Scholar (<http://scholar.google.com>) up until December 2018. Persian and English terms used for literature search were 'antibiotic resistance', '*C. difficile*', '*C. perfringens*', '*C. tetani*', '*C. botulinum*' and 'Iran'. To ensure a complete literature search and to avoid missing

any relevant studies, a manual search in the bibliography of included articles was conducted to identify additional eligible studies.

2.2. Selection criteria

Collected studies were evaluated in the Endnote reference management software based on inclusion and exclusion criteria to find articles on the prevalence of antibiotic-resistant *Clostridium* species in Iran. Our selection criteria were clostridial antimicrobial resistance studies limited to Iran and published in both Persian and English languages; however, studies with incomplete information, and those that assessed the rates of incidence of infections and antibiotic resistance genes were excluded. There was no limitation regarding the type of specimens (animal or human sources) and type of reports, except for duplicate and non-original articles such as abstracts of congress articles, reviews and case reports. Eligibility of selected articles was evaluated by two authors independently and then reviewed again to extract desired data from each study.

2.3. Data extraction

The Newcastle-Ottawa Quality Assessment Scale (NOS) was used to evaluate the quality of included studies according to selection, comparability and outcome criteria (data not shown). As shown in Table 1, the following data were extracted from the eligible articles: location of the study, publication year of the study, specimen type, *Clostridium* species, number of isolated bacteria, methods used for assessing antimicrobial susceptibility and number of bacterial isolates resistant to different antibiotics.

2.4. Statistical analysis

All statistical analysis including meta-analysis of data on the prevalence of antibiotic resistance patterns for each *Clostridium* species was performed using the Comprehensive Meta-Analysis (CMA) software version 2.2 (Biostat, Englewood, NJ). The results were expressed as a percentage and with 95% confidence intervals (95% CIs). Inter-study heterogeneity was assessed using I^2 statistic and expressed as a percentage (%). A random-effects model was chosen for meta-analysis when I^2 value was >25%. Publication bias was evaluated by exploring funnel plots.

3. Results

3.1. Literature search

As shown in Figure 1, an electronic search in PubMed, Scopus and Google Scholar databases yielded 1,026 articles. Of these, 1,005 articles did not meet our

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

Province/City	Year	Sample type	Clostridium species	Strain (n)	AST	Antibiotic resistance (n)																Ref
						AMP	CHL	CIP	CLI	DOX	ERY	GEN	MTZ	NAL	TET	VAN	PEN	TMP/SXT	AMX	IPM	CRO	
Chaharmahal va Bakhtiari	2013	Animal (Stool)	C. difficile	90	Disk diffusion	ND	ND	30	90	ND	81	ND	ND	ND	6	72	ND	ND	ND	ND	ND	[23]
Isfahan Shahrekord	2013	Food	C. difficile	5	Disk diffusion	1	0	4	5	0	2	4	0	5	2	0	ND	ND	ND	ND	ND	[24]
Isfahan Chaharmahal va Bakhtiari Khuzestan	2013	Food	C. difficile	2	Disk diffusion	1	0	0	2	0	0	2	0	2	0	0	ND	ND	ND	ND	ND	[25]
Isfahan Khuzestan	2012	Food	C. difficile	13	Disk diffusion	7	0	10	12	0	8	13	0	13	4	0	ND	ND	ND	ND	ND	[26]
Mashhad	2012	Food	C. perfringens	6	Disk diffusion	0	ND	ND	ND	ND	6	6	ND	ND	ND	ND	0	ND	ND	ND	ND	[27]
Tabriz	ND	Food	C. perfringens	40	Disk diffusion	11	0	ND	ND	ND	12	21	ND	21	32	4	8	7	12	ND	ND	[28]
Tabriz	ND	Human (Stool)	C. perfringens	79	E-test	ND	4	ND	13	ND	ND	ND	9	ND	ND	ND	7	ND	ND	30	2	[29]
Tabriz Tehran	2015 2010–2016	Food Human	C. difficile C. difficile	8 46	Disk diffusion Disk diffusion	ND 19	1 ND	5 44	7 42	ND ND	4 ND	ND ND	0 35	8 ND	3 30	0 6	ND ND	ND ND	ND ND	ND 14	ND 32	[30] [31]
Tehran	2015	Human (Stool)	C. difficile	35	Disk diffusion	ND	ND	ND	8	ND	20	ND	10	ND	25	7	ND	ND	ND	ND	ND	[32]
Tehran	2010–2011	Human (Stool)	C. difficile	75	Agar dilution	ND	ND	ND	67	ND	43	ND	4	ND	ND	6	ND	ND	ND	ND	ND	[33]
Tehran	2002–2006	Human (Stool)	C. difficile	57	Disk diffusion	ND	0	15	35	ND	ND	57	0	ND	8	0	ND	ND	ND	ND	0	[34]
Tehran	ND	Human (Stool)	C. difficile	86	Agar dilution	ND	ND	83	ND	ND	ND	ND	4	ND	ND	ND	ND	ND	ND	41	ND	[35]
Tehran	ND	Animal (Stool)	C. perfringens	46	Disk diffusion	ND	0	7	0	ND	6	5	ND	ND	0	ND	29	23	5	ND	ND	[20]

Abbreviations: AMP-ampicillin; CHL-chloramphenicol; CIP-ciprofloxacin; CLI-clindamycin; DOX-doxycycline; ERY-erythromycin; GEN-gentamicin; MTZ-metronidazole; NAL-nalidixic acid; TET-tetracycline; VAN-vancomycin; PEN-penicillin; TMP/SXT-trimethoprim-sulfamethoxazole; AMX-amoxicillin; IPM-ipinenem; CRO-ceftioxone; ND-not determined; AST-antimicrobial susceptibility testing.

Figure 1. Flowchart of the literature search.

inclusion criteria after screening of the titles and abstracts. The reasons for exclusion were that they were duplicates, those that reported bacterial antibiotic resistance in non-*Clostridium* species, those that reported antibiotic resistance of *Clostridium* species from other countries, and those that were not original articles. For the next step, 21 full-text articles were evaluated for eligibility. Articles with insufficient data and simultaneous publication in both Persian and English languages were excluded. After adding additional identified studies from the reference lists of eligible articles, 14 studies were included in our systematic review and meta-analysis.

3.2. Study characteristics

The characteristics of 14 eligible studies are presented in Table 1. Included studies were reported from Chaharmahal va Bakhtiari, Isfahan, Khuzestan, Mashhad, Tabriz and Tehran. *C. difficile* and *C. perfringens* were the most common *Clostridium* species isolated from human and animal stools, and from different foods. Disk diffusion was the most commonly employed test to determine the prevalence of antibiotic-resistant *Clostridium* species in Iran, followed by Agar dilution and E-tests. Characteristics of *C. difficile* and *C. perfringens* antibiotic resistance were also reported in included studies as shown in Table 1. However, we did not find any report about the antibiotic resistance patterns of other *Clostridium* species in Iran. Finally, asymmetric distribution of studies in Figures 2(b) and 3(b) indicated potential presence of publication bias in the current meta-analysis.

3.3. Characteristics of *C. difficile* antibiotic resistance

In total, 10 studies reported *C. difficile* antibiotic resistance in Iran (Table 1). Fixed-effects model ($I^2 < 25\%$) were used for pooling the data on the prevalence of *C. difficile* resistance to ampicillin, chloramphenicol, doxycycline and nalidixic acid antibiotics, and a random-effects model ($I^2 > 25\%$) was used for the meta-analysis of the rest of drugs. Antibiotic resistance patterns of *C. difficile* in Iran were as follow: 42.8% (95% CI: 31.4–55.1; $I^2 = 0\%$; $p = 0.64$) to ampicillin, 6.2% (95% CI: 2–17.8; $I^2 = 0\%$; $p = 0.51$) to chloramphenicol, 69.5% (95% CI: 40.3–88.5; $I^2 = 90.9\%$; $p = 0.00$) to ciprofloxacin, 84.3% (95% CI: 63.5–94.3; $I^2 = 87.1\%$; $p = 0.00$) to clindamycin, 0% to doxycycline, 61.5% (95% CI: 42.6–77.5; $I^2 = 77.9\%$; $p = 0.00$) to erythromycin, 93.5% (95% CI: 75–98.6; $I^2 = 26.8\%$; $p = 0.25$) to gentamicin, 10.7% (95% CI: 2.8–33.4; $I^2 = 90\%$; $p = 0.00$) to metronidazole (Figure 2(a)), 92.9% (95% CI: 75.4–98.2; $I^2 = 0\%$; $p = 0.87$) to nalidixic acid, 32.5% (95% CI: 14–58.8; $I^2 = 89.3\%$; $p = 0.00$) to tetracycline, 12.5% (95% CI: 3.2–38.2; $I^2 = 92.3\%$; $p = 0.00$) to vancomycin, 39.6% (95% CI: 24.3–57.3; $I^2 = 72.2\%$; $p = 0.05$) to imipenem and 14.3% (95% CI: 0.1–97.5; $I^2 = 93.1\%$; $p = 0.00$) to ceftriaxone. In addition, *C. difficile* antibiotic resistance rates to levofloxacin, ertapenem, piperacillin/tazobactam, rifampicin, fidaxomicin, tigecycline, linezolid, fusidic acid, cefoperazone, cefepime, kanamycin, colistin, ceftazidime, amikacin, moxifloxacin and cefotaxime were 93.4%, 58.7%, 56.5%, 14.2%, 8.5%, 14.2%, 8.5%, 11.4%, 3.5%, 10.5%, 100%, 100%, 76%, 76.5%, 67.9% and 95%, respectively.

Figure 2. Meta-analyses of the prevalence of *C. difficile* resistant to metronidazole in Iran. (a) Forest plot and (b) Funnel plot.

3.4. Characteristics of *C. perfringens* antibiotic resistance

Four studies reported *C. perfringens* antibiotic resistance in Iran (Table 1). A fixed-effects model ($I^2 < 25\%$) was used for the meta-analysis of data on the prevalence of resistance of *C. difficile* to ampicillin, chloramphenicol, ciprofloxacin, metronidazole, nalidixic acid, vancomycin, imipenem and ceftriaxone antibiotics. The prevalence of *C. perfringens* resistance to different antibiotics was as follows: 25.8% (95% CI: 15–40.6; $I^2 = 10.4\%$; $p = 0.29$) to ampicillin, 3.7% (95% CI: 1.6–8.7; $I^2 = 0\%$; $p = 0.39$) to chloramphenicol, 15.2% (95% CI: 7.4–28.6; $I^2 = 0\%$; $p = 1.0$) to

ciprofloxacin, 6% (95% CI: 0.4–50.7; $I^2 = 75\%$; $p = 0.04$) to clindamycin, 32.9% (95% CI: 10.4–67.3; $I^2 = 80.1\%$; $p = 0.00$) to erythromycin, 45.4% (95% CI: 10.1–86.1; $I^2 = 89.7\%$; $p = 0.00$) to gentamicin, 0% to metronidazole, 52.5% (95% CI: 37.3–67.3; $I^2 = 0\%$; $p = 1.0$) to nalidixic acid, 19.5% (95% CI: 0.1–98.8; $I^2 = 93.7\%$; $p = 0.00$) to tetracycline, 10% (95% CI: 3.8–23.8; $I^2 = 0\%$; $p = 1.0$) to vancomycin, 21.8% (95% CI: 5.4–57.8; $I^2 = 92\%$; $p = 0.00$) to penicillin (Figure 3(a)), 32.1% (95% CI: 9.4–68.4; $I^2 = 89.1\%$; $p = 0.00$) to trimethoprim-sulfamethoxazole, 19.3% (95% CI: 6.5–44.9; $I^2 = 78.2\%$; $p = 0.03$) to amoxicillin, 38% (95% CI: 28–49.1; $I^2 = 0\%$; $p = 1.0$) to imipenem

Figure 3. Meta-analyses of the prevalence of *C. perfringens* resistant to penicillin in Iran. (a) Forest plot and (b) Funnel plot.

and 2.5% (95% CI: 0.6–9.6; $I^2 = 0\%$; $p = 1.0$) to ceftriaxone. On the other hand, *C. perfringens* resistance rates to different antibiotics including cloxacillin, cephalexin, oxacillin, cephalothin, bacitracin and colistin were 100%, 0%, 45.6%, 8.6%, 89.1% and 40%, respectively.

4. Discussion

The use of antibiotics, particularly clindamycin, expanded- and extended-spectrum cephalosporins along with fluoroquinolones, ampicillin and amoxicillin, are associated with a high risk of CDI [11,12]. In the present study, clindamycin resistance was found in 84.3% of *C. difficile* isolates from clinical and non-clinical specimens of Iran. Antibiotic resistance of clinical isolates of *C. difficile* reported in different countries was as follows: Japan (87.7%), Germany (0%), Sweden (65%),

France (34.8%), Spain (74%), Korea (81%), China (88.1%), Poland (38%), Hungary (31%), United States (36%), New Zealand (61%), Czech Republic (10%), Brazil and Israel (0%) [12]. Differences in the statistics may be due to differences in the year of study, susceptibility tests, geographic area and strain type of *C. difficile*, as antibiotic resistance rate in epidemic strains of *C. difficile* is higher than non-epidemic strains [12]. *C. difficile* resistance to β -lactam antibiotics, *i.e.* penicillins, cephalosporins and carbapenems, were variable in the current meta-analysis. Resistance rate to cephalosporins including ceftriaxone (14.3%), cefoperazone (3.5%) and cefepime (10.5%) were low, except for ceftazidime (76%) and cefotaxime (95%). Also, ampicillin (42.8%), imipenem (39.6%) and ertapenem (58.7%) resistance rates were high in *C. difficile* isolates. In Iran, resistance rates to quinolones including ciprofloxacin (69.5%), nalidixic acid (92.9%), levofloxacin (93.4%) and moxifloxacin

(67.9%) were high in *C. difficile* strains. Similar results regarding moxifloxacin resistance rate were observed in China (61.8%), Korea (62.6%), Germany (68%), Czech Republic (100%) and Poland (100%), while moxifloxacin resistance rates in Japan (0%), Sweden (15%), France (8%), Spain (43%), Hungary (41.2%), United States (36%), New Zealand (0%), Brazil (8%) and Israel (4.7%) were low [12]. It has been suggested that excessive use of fluoroquinolone antibiotics is associated with the emergence of hypervirulent *C. difficile* 027/BI/NAP1 strains [13]. Therefore, owing to the fact that quinolones' resistance rate was high in Iran, caution should be exercised in prescribing these drugs and determining *C. difficile* strains' ribotypes is recommended. Metronidazole and vancomycin are two main first-line antibiotics which are used to treat primary and recurrent CDI [14,15]. The prevalence of *C. difficile* resistance to metronidazole (10.7%) (Figure 2(a)) in Iran was lower than Israel (20.2%); however, it was higher than Japan (0%), Germany (0%), Sweden (0%), France (0%), Spain (0%), Korea (0%), China (0%), Poland (0%), Hungary (0%), United States (0%), New Zealand (0%), Czech Republic (0%) and Brazil (0%) [12]. Vancomycin is another important drug which should be used in severe diseases due to *C. difficile*. Vancomycin resistance rate among *C. difficile* isolates in Iran was 12.5%. This rate was lower than those reported from United States (13.2%), Poland (41.5%), Hungary (29.5%), Brazil (58%) and Israel (31.5%), while being higher than those of Japan (0%), Germany (0%), Sweden (0%), France (0%), Spain (0%), Korea (0%), China (0%), New Zealand (0%) and Czech Republic (0%) [12]. Fidaxomicin, tetracyclines, rifamycins and chloramphenicol are other therapeutic options for CDI [13]. The prevalence of fidaxomicin-resistant *C. difficile* isolates is rare [13]. However, *C. difficile* resistance to fidaxomicin (8.5%) was high in Iran. Therefore, fidaxomicin use should be controlled in order to prevent the emergence of resistant strains under selective pressure. In our study, tetracycline resistance rate (32.5%) was lower than China (62.7%) and higher than Japan (0%), Germany (0%), Sweden (7.5%), France (0%), Spain (0%), Korea (0%), Poland (0%), Hungary (0%), United States (6.7%), New Zealand (0%), Czech Republic (0%), Brazil (0%) and Israel (0%) [12]. However, doxycycline (0%) and tigecycline (14.2%) resistance rates were low in Iran. Today, it is recommended that tigecycline can be used as an alternative antibiotic for severe CDI therapy [15]. The prevalence of rifampicin-resistant *C. difficile* varied from 0% to 65% in different countries [12]. In Iran, *C. difficile* resistance rates to rifampicin was 14.2%. Also, the incidence of chloramphenicol-resistant *C. difficile* isolates was estimated to be rare. The prevalence of *C. difficile* resistance to chloramphenicol in Iran was 6.2%. Similar results were reported in Europe (3.7%) [16].

High-dose penicillin is the most common antibiotic therapy used to treat *C. perfringens*-related soft

tissue infections in humans [1]. The prevalence of penicillin-resistant *C. perfringens* strains is rare and the results of different studies from Canada (0%) [17], New Zealand (0%) [18] and Brazil (0%) [19] confirmed this. However, penicillin-resistant *C. perfringens* rate was high in Iran (21.8%) (Figure 3(a)). The difference in the type of obtained samples could be a possible reason for this difference. *C. perfringens* is present in the gastrointestinal tracts of animals and humans and is responsible for food-borne gastrointestinal infections that are resistant to several antibiotics [7]. Therefore, there are concerns about the transfer of resistance genes through mobile genetic elements to other gut microflora and food-borne bacteria [7]. A frequently used antibiotic to prevent or treat clostridiosis in the Iranian poultry industry is bacitracin [20]. Our study showed that 89.1% of *C. perfringens* isolates in Iran were resistant to bacitracin. A similar result was reported by Silva *et al.* in Brazil [19]. However, the bacitracin resistance rate was 3% in Sweden and 15% in Denmark [21]. Only a few studies evaluating bacitracin resistance were found in our search and the negligible use of bacitracin in the mentioned countries is a possible reason for different results. The prevalence of tetracycline-resistant *C. perfringens* strains varied from 10% to 76% and it is the most common resistance in *C. perfringens* strains [22]. In the current study, 19.5% of *C. perfringens* isolates were resistant to tetracycline in Iran. Drug resistance rate of *C. perfringens* isolates, regardless of origin, to chloramphenicol (3.7%), ciprofloxacin (15.2%), clindamycin (6%), metronidazole (0%), vancomycin (10%), ceftriaxone (2.5%), cephalixin (0%) and cephalothin (8.6%) were low. These high degrees of susceptibility can be attributed to the negligible use of the mentioned antibiotics in controlling the clostridial diseases in humans and domestic animals in Iran, small number of studies assessing resistance and origins of analyzed samples.

5. Conclusion

Our results revealed that the prevalence of resistance to metronidazole and vancomycin, as first-line therapies, among *C. difficile* isolates was low in Iran. In addition, these bacteria showed the same resistance rates to fidaxomicin, tetracyclines (except tetracycline), rifampicin and chloramphenicol antibiotics. Therefore, the aforementioned antibiotics have a good efficacy and can still be used for CDI therapy in Iran. However, the resistance rate of *C. perfringens* to the most commonly used antibiotics *i.e.* penicillin (in human severe infections) and bacitracin and tetracycline (in clostridial diseases in domestic animals) was high. Accordingly, these antibiotics are not recommend in clostridial diseases of

humans and domestic animals in Iran. We also propose determining the hypervirulent ribotypes of *C. difficile* strains and bacterial resistance mechanisms of *C. difficile* and *C. perfringens* strains to various antibiotics in different cities of Iran. Finally, investigation of the prevalence of *C. tetani* and *C. botulinum* resistance rates to different antibiotics, especially metronidazole and penicillin, is warranted to allow a better control of *Clostridium*-related diseases in Iran.

Disclosure statement

No potential conflict of interest was reported by the authors.

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