

Research Paper

Antibiotic Susceptibility Profile of Clostridium Difficile Bacteria Isolated from Older Residents of a Nursing Home in Iran



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**ABSTRACT**

**Objectives** Clostridium difficile (C. diff) is a gram-positive anaerobic bacterium known as the most common cause of nosocomial diarrhea in nursing homes. The antibiotic susceptibility profile is the basic way for successful treatment due to antimicrobial resistance. This present study aims to assess the antibiotic susceptibility profile of C. diff isolated from older residents of a nursing home in Tehran, Iran.

**Methods & Materials** Forty-two isolates of C. diff were used in this study collected from 289 residents of Kahrizak Nursing Home. Antibiotic susceptibility testing was conducted by using disk-diffusion method, agar dilution method, and Epsilonometer test (E-test).

**Results** All C. diff strains were susceptible to Metronidazole, Vancomycin, Rifampicin, Linezolid and Tigecycline. By using the disk-diffusion method, the highest rate of resistance was related to Clindamycin (100%), Levofloxacin (96.2%), Imipenem (81%), Azithromycin (61%) and Erythromycin (54.8%). All C. diff strains were susceptible to Metronidazole under E-test. Furthermore, 100% and 59.5% of strains were susceptible to Vancomycin and Erythromycin, respectively under agar dilution test.

**Conclusion** C. diff strains are sensitive to Vancomycin and Metronidazole. These two antibiotics can be used to treat C. diff infections in older adults. The disk diffusion method can be used as a screening test to determine antibiotic resistance.

**Extended Abstract**

**1. Introduction**

**C**lostridium difficile (C. diff) is a gram-positive, obligate anaerobic, and spore-forming bacillus that was identified in 1935 as part of the flora of healthy infants. This bacterium is an important human pathogen that has been introduced as a causative agent of antibiotic-associated diarrhea and pseudomembranous colitis [1, 2]. This

bacterium is the most common cause of infectious diarrhea in nursing homes, which in recent years. About 3 million cases of diarrhea and colitis are reported annually. Mortality associated with C. diff infectious diarrhea is estimated at 17%, which is higher in the elderly [5, 6]. Colonization by C. diff isolates producing toxins has been reported in nursing home residents ten times more than in those dwelling in the community [7]. It is reported that about 57% of the residents of nursing homes can be carriers of this bacterium [7, 8]. In Iran, there is also the problem of irrational use or overuse of antibiotics [14]. The resistance to these

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drugs is increasing in the world and there are no reports of their usage among the residents of nursing homes in Iran. Antibiotic susceptibility profile is the most basic way for successful treatment due to antimicrobial resistance. This study aims to evaluate the antibiotic susceptibility profiles of *C. diff* bacteria isolated from the elderly living in a nursing home in Tehran, Iran.

## 2. Methods & Materials

In this study, 289 fecal samples from residents of Kahrizak nursing home in Tehran, Iran who were over 60 years old (ranging from 60 to 101) and hospitalized for at least one month were examined for antibiotic susceptibility (44.3% male and 55.7% female). of 289 samples, 42 (14.5%) tested positive for *C. diff*.

### Antibiotic susceptibility test by disk diffusion method

Discs of antibiotics were metronidazole, vancomycin, imipenem, clindamycin, chloramphenicol, azithromycin, linezolid, tigecycline, teicoplanin, rifampin, erythromycin and amoxicillin-clavulanic acid (Mast Group Ltd, UK). Isolates were first cultured in Cycloserine-cefoxitin fructose Agar (CCFA) medium and 1 McFarland dilution in physiological saline was prepared from each grown sample. Then, swab was used for superficial culture on Bru-

cella agar medium (Merck Co.) with 5% sheep blood and 1 µg/mL vitamin K (Sigma Co.). The tested disks were then placed on the culture medium and incubated for 48 hours at 37 ° C in an anaerobic jar containing GasPak type A (Merck Co.). Disk diffusion of each drug was interpreted according to published articles [15-21].

### Antibiotic susceptibility test by agar dilution method and E-test

Antibiotic susceptibility testing was performed with agar dilution method for vancomycin and erythromycin antibiotics (Sigma Co.) and Epsilometer test (E-test) for metronidazole antibiotic (Liofilchem Co., Italy), according to the protocol of Clinical and Laboratory Standards Institute 2018 (CLSI 2018). First, 0.5 McFarland dilution was prepared from isolates grown in CCFA culture medium. Then, 1 microliter of 0.5 McFarland dilution was cultured on Brucella agar medium with different dilutions of antibiotics, containing 5% sheep blood and vitamin K (1 µg/mL). For E-test, 1 McFarland dilution was cultured on by swap on Brucella agar medium containing 5% sheep blood and 1 µg/mL vitamin K. Next, metronidazole strip was placed on the culture. The cultured plates were then incubated for 48 hours at 37 ° C in an anaerobic jar containing GasPak type A [22]. The results were interpreted according to CLSI 2018 guidelines.

**Table 1.** Prevalence of antibiotic susceptibility by disk diffusion method in *C. diff* isolates

Antibiotic Disk	Susceptible No. (%)	Resistant, No. (%)	Range zone, mm
Clindamycin 2µg	0 (0.00)	42 (100)	0
Teicoplanin 30µg	40 (95.2)	2 (4.8)	16-32
Tigecycline 15µg	42 (100)	0 (0.00)	24-48
Levofloxacin 5µg	2 (4.8)	40 (96.2)	0-28
Metronidazole 5µg	42 (100)	0 (0.00)	32-56
Vancomycin 30µg	42 (100)	0 (0.00)	20-32
Imipenem 10µg	8 (19)	34 (81)	0-32
Rifampicin 5µg	42 (100)	0 (0.00)	16-48
Azithromycin 15µg	16 (39)	25 (61)	0-32
Erythromycin 15µg	19 (45.2)	23 (54.8)	0-40
Linezolid 30µg	42 (100)	0 (0.00)	24-48
Amoxicillin/clavulanic acid 30µg	35 (83.3)	7 (17.7)	20-36
Chloramphenicol 30µg	32 (76)	10 (24)	0-34

**Table 2.** Prevalence of antibiotic susceptibility by agar dilution method and E-test in *C. diff* isolates

Antibiotic	MIC Range (µg/ml)	Resistant, NO. (%)	Break Points (µg/ml)
Metronidazole	0.016-0.94	0 (0.00)	≥ 32
Vancomycin	0.125-0.5	0 (0.00)	≥2
Erythromycin	0.5-16	17 (40.5)	≥8

### 3. Results

*C. difficile* isolates were 100% resistant to clindamycin; 96.2% to levofloxacin; 54.8% to erythromycin; 81% to imipenem, and 61% to azithromycin by disk diffusion method. The antibiotic susceptibility of *C. diff* by disk diffusion method was 95.2% to teicoplanin; 76% to chloramphenicol, and 83.3% to amoxicillin-clavulanic acid. 100% of the isolates were sensitive to metronidazole, vancomycin, linezolid, tigecycline, and rifampin while 45.2% were sensitive to erythromycin (Table 1). All *C. diff* isolates were sensitive to metronidazole using E-test. By agar dilution method, all *C. diff* isolates were sensitive to vancomycin, while 59.5% were sensitive to erythromycin (Table 2).

### 4. Conclusion

The present study was performed to determine the susceptibility of *C. diff* to common antibiotics such as vancomycin, metronidazole and erythromycin in residents of nursing homes in Tehran, Iran. Inappropriate use of antibiotics is one of the effective factors in causing diarrhea by CDI; hence, it is necessary to stop the use of antibiotics for treatment [23]. Antibiotics are one of the most widely used drugs in the world and are extremely valuable in terms of general health. However, the effect of antibiotics in the community and the risk of resistance may also be affected by how the patient uses antibiotics [11]. Antibiotic resistance plays an important role in the emergence of new strains, and transmission of genetic elements can occur between *C. diff* strains or between *C. diff* and other bacteria, where increase resistance [23, 38].

The results were the same for metronidazole and vancomycin antibiotics by using disk diffusion method, agar dilution method and E-test. *C. diff* strains were sensitive to metronidazole, vancomycin, linezolid, tigecycline, teicoplanin, rifampin and amoxicillin-clavulanic acid. Therefore, the disk diffusion method can be a suitable method for testing the antibiotic susceptibility of *C. diff*. Study limitations included financial constraints and difficulty sampling the elderly. Due to our financial constraints, only three metro-

nidazole, vancomycin, and erythromycin antibiotics were selected for agar dilution test and E-test.

*Clostridium difficile* isolates were sensitive to vancomycin and metronidazole antibiotics using disk diffusion and agar dilution methods. These antibiotics can be used in cases of *C. diff* infection in the elderly. However, the use of vancomycin by injection is not recommended for outpatients. On the other hand, the disk diffusion method, as a simple and inexpensive screening method can be used to determine the antibiotic resistance of *C. diff*, and resistant isolates be confirmed by Minimum Inhibitory Concentration parameter.

### Ethical Considerations

#### Compliance with ethical guidelines

This study was approved by Ethical Committee of Tarbiat Modares University, Tehran.

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#### Authors' contributions

All authors equally contributed in preparing this article.

#### Conflicts of interest

The authors declared no conflict of interest.

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