Determination of fosfomycin susceptibility in carbapenemase-producing K. pneumoniae strains isolated prior to clinical use of the intravenous formulation in Turkey

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ABSTRACT

Objectives: The incidence of infections caused by carbapenem-resistant Enterobacteriaceae has increased worldwide. Limitations in the development of new antimicrobial agents have led clinicians to reconsider the clinical efficiency of old antibiotics, such as intravenous formulation of fosfomycin, in the treatment of multidrug-resistant Gram-negative bacterial infections. We investigated the fosfomycin susceptibility of carbapenemase-producing Klebsiella pneumoniae strains isolated prior to the clinical use of the intravenous formulation of fosfomycin in Turkey.

Materials and Methods: A total of the 155 K. pneumoniae isolates which previously characterized at the molecular level for their carbapenemase were included in this study. The minimum inhibitory concentration of fosfomycin was determined by the agar dilution method.

Results: Overall, 65.1% of the isolates were susceptible to fosfomycin. The MIC50 and MIC90 values were 32 and 256 mg/L, respectively. According to our results, at least two-third of carbapenemase-positive K. pneumoniae are susceptible to fosfomycin.

Conclusions: Although, the susceptibility of fosfomycin, which has just been put into the clinical use of intravenous formulation in Turkey, is not very high, it can be considered as an alternative adjunct antimicrobial for the treatment of systemic infections caused by carbapenemase-positive K. pneumoniae isolates.

Keywords: Fosfomycin susceptibility, Carbapenemase-producing Klebsiella pneumoniae

1.INTRODUCTION

The emergence and spread of carbapenem-resistant Enterobacteriaceae (CRE) have become a global health problem during the last decade. Generally, CRE isolates are multi-drug-resistant, and the most prevalent mechanisms of carbapenem resistance in Enterobacteriaceae have been associated with the production of carbapenem-hydrolyzing enzymes. Different types of class A (e.g. KPC, GES), class B (e.g. NDM, IMP, VIM) and class D (e.g. OXA-48-like) enzymes have been identified in Enterobacteriaceae [1-3]. Treatment options of infections caused by CRE are challenging due to limited antimicrobials available. Therefore, it is very important to develop new and effective therapeutic strategies [4].

The limitations in the development of new antimicrobial agents have led physicians to reassess the clinical efficacy of old antibiotics, such as fosfomycin, in the treatment of multidrug-resistant Gram-negative bacterial infections. Fosfomycin is an old broad-spectrum antibiotic and active against both Gram-positive and Gram-negative bacteria [5]. Oral fosfomycin is commonly used for the treatment of uncomplicated urinary tract infections. The intravenous formulation has been available in many European countries (United Kingdom, Spain, France, Germany, Austria, Greece, etc.) and some Asian countries (Japan) for the treatment of systemic infections for several years [6]. In some European studies, the use of IV fosfomycin...
had a good effect on clinical and microbiological outcome in patients infected with various carbapenem-resistant Klebsiella pneumoniae strains. Fosfomycin seems to be an alternative agent for the treatment of such difficult to treat infections [7]. However, one of the main problems with this drug is the potential emergence of resistance during monotherapy. Therefore, it is recommended to use fosfomycin in combination with other antimicrobial agents (such as carbapenems, aminoglycosides, colistin, and tigecycline) in serious infections. Although, various combination regimens are used for the treatment of CRE infections, the clinical evidence is mainly based on case reports and retrospective studies. Therefore, more studies are needed to determine fosfomycin efficiency in the treatment of systemic infections [4, 6].

The intravenous formulation of fosfomycin was introduced in December 2018 for the treatment of systemic infections in Turkey. In this study, we investigated the fosfomycin susceptibility of carbapenemase-producing K. pneumoniae strains isolated prior to the commercial availability of the intravenous formulation of fosfomycin.

2. MATERIALS and METHODS

Study isolates

The Klebsiella pneumoniae isolates used in this study were collected from various clinical samples (bronchoalveolar lavage, deep tracheal aspirate, sputum, blood, urine, wound) of infected patients between January 2015 and December 2017 from hospitalized patients at Marmara University Hospital, Istanbul, Turkey. All of the non-duplicate isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (VITEK-MS, bioMerieux, Marcy l’Etoile, France). Carbapenem susceptibility was initially determined using an automated antimicrobial susceptibility test system (VITEK2, bioMerieux, Marcy-l’Etoille, France). Minimal inhibitory concentrations (MICs) of imipenem, meropenem, and ertapenem were determined by the gradient diffusion strip test (Etest®, bioMérieux, Marcy l’Etoile, France) and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [8]. Molecular detection of the different carbapenemase genes was carried out by in-house polymerase chain reaction (PCR) using the specific primers for genes encoding blaVIM, blaIMP, blaNDM, blaKPC, and blaOXA-48 [9-12]. A total of 155 carbapenemase-positive K. pneumoniae isolates confirmed by polymerase chain reaction (PCR) method were selected for fosfomycin susceptibility testing. We excluded isolates carrying more than one type of carbapenemases.

Fosfomycin susceptibility testing

Fosfomycin trometamol was obtained from Drogsan (Istanbul, Turkey) and glucose-6-phosphate (G6P) from Sigma Aldrich (St. Louis, MO, USA). The agar dilution method was used to determine the susceptibility of fosfomycin on Mueller Hinton agar (MHA) (Becton Dickinson, New Jersey, USA) supplemented with 25 mg/L G6P, according to the EUCAST guidelines. The MHA plates containing fosfomycin in concentrations from 0.06 to 256 mg/L were prepared, surface-inoculated with 10⁴ CFU/mL and allowed to dry. The plates were incubated for 16 to 20 h at ambient air at 35°C. Results of antibiotic susceptibility testing were interpreted using the EUCAST criteria, where ≤ 32 mg/L is considered as susceptible and > 32 mg/L as resistant [8]. Escherichia coli ATCC 25922 was used as the quality control strain.

Statistical Analysis

The differences in fosfomycin susceptibility between the groups were compared using the Fisher’s exact test. The differences between the groups were considered significant if the p-values were smaller than 0.05.

3. RESULTS

Carbapenemase-positive isolates expressed OXA-48 (n = 82), NDM (n = 50), IMP (n = 11), KPC (n = 7), and VIM (n = 5) carbapenemase. The isolates were resistant at least one of the carbapenems (ertapenem, imipenem, and meropenem) according to the EUCAST guidelines. The MIC₅₀ and MIC₉₀ values were 32 and 256 mg/L, respectively. Among the various carbapenemase types, KPC-positive isolates had the lowest MIC₅₀ and MIC₉₀ values (Table I).
Table I. Fosfomycin susceptibility in various types of carbapenem producing K. pneumoniae

| Carbapenemase type | Susceptible (n) | Resistant (n) | MIC_{50} (mg/L) | MIC_{90} (mg/L) | MIC Range (mg/L) | Fosfomycin susceptibility (%) | p |
|-------------------|----------------|--------------|----------------|----------------|------------------|--------------------------|---|
|                   | 2* | 4* | 8* | 16* | 32* | 64* | 128* | 256* |             |
| OXA-48-like (n = 82) | 1  | 13 | 14 | 26 | 12 | 3 | 13 | 32 | 256 | 2->256 | 65.8 | 1 |
| NDM (n = 50) | 1  | 2  | 14 | 16 | 6  | 5 | 6  | 32 | 256 | 4->256 | 66  | 1 |
| IMP (n = 11) | 1  | 1  | 1  | 1  | 2  | 2 | 1  | 32 | 128 | 4->256 | 54.5 | 1 |
| KPC (n = 7) | 1  | 2  | 1  | 1  | 1  | 1 | 8  | 64 | 2-256 | 71.4 | 0,52 |
| VIM (n = 5) | 3  | 1  | 1  | 8  | 128 | 8-256 | 60  | 1 |
| TOTAL (n = 155) | 2  | 2  | 21 | 46 | 21 | 11 | 22 | 32 | 256 | 2->256 | 65.1 |

MIC: Minimal inhibitory concentration(mg/L)

* The MICs given in the table are the susceptibility breakpoint for fosfomycin recommended by EUCAST.

4. DISCUSSION

Antimicrobial resistance in Gram-negative microorganisms is a global problem. In particular, carbapenem-resistant K. pneumoniae has spread all over the world in the last decade and represents one of the most critical challenges to the antimicrobial therapy of such infections. Because of the shortage of new antimicrobials, clinicians have begun to re-evaluate already existing antibiotics such as fosfomycin. Fosfomycin is a small, hydrophilic agent with almost negligible serum protein binding. It is a broad-spectrum agent that is active against multidrug-resistant bacterial pathogens (MRSA, VRE, ESBL, CRE) and is excreted unchanged in the urine, reaching high urinary concentrations after administration of the 3-g single dose [13]. Therefore, the oral formulation is generally safe in uncomplicated urinary tract infections. Rapid penetration and distribution of fosfomycin into tissues such as soft tissue, lungs, bone, and cerebrospinal fluid is an important feature for preference in the treatment of serious infections caused by MDR-pathogens [7]. While intravenous fosfomycin has been prescribed for a wide variety of infections in many countries for more than 40 years, this formulation has been introduced into Turkey in late 2018. Therefore, clinical and in-vitro susceptibility studies are limited in this country [14, 15]. Fosfomycin susceptibility among CRE ranges between 42 and 98%, according to results of studies conducted in different countries [16-25]. We found a 65.1% susceptibility rate of fosfomycin against 155 carbapenemase-positive Klebsiella pneumoniae isolates in our study. Fosfomycin susceptibility rate found in our study was lower when compared to previous studies, mainly because most of these studies were performed only on KPC-producing strains and were evaluated according to EUCAST criteria (susceptible ≤ 64 mg/L) before 2011. Susceptibility data of CRE carrying other carbapenemases is also limited. Our study results will contribute to the literature in account of having the results for the most frequently observed five carbapenemase types (OXA-48, NDM, KPC, IMP, VIM) and evaluating them according to the current EUCAST fosfomycin breakpoint.

After intravenous administration of fosfomycin, mean peak serum concentrations commonly range from 200 to 600 mg/L, according to several studies [13]. These values are well above the clinical breakpoints determined by EUCAST for Enterobacteriaceae (susceptible ≤ 32 mg/L). Also, fosfomycin MICs of 85.8% of our study isolates had ≤ 128 mg /L and were below the achievable serum concentration values. The correlations of PD parameters (concentration and/or time-dependent activity) with antibacterial activity of fosfomycin have not yet been clarified. Although it is known that even high doses (20-24 g/day) are well-tolerated, no definitive treatment protocol has been established yet. However, it should be remembered that resistance may develop during the use of fosfomycin monotherapy, and resistance development has been reported in approximately 3.4% during the treatment in some studies [6, 25]. Therefore, in serious infections, it is recommended to use it in combination with other antimicrobial agents such as carbapenems, aminoglycosides, colistin or...
tigecycline [26, 27]. In some studies, treatment success rates of > 80% have been achieved with the combined use of fosfomycin with other antimicrobial agents in the treatment of serious infections [6, 28-31].

The limitation of this study is that excluded isolates carrying more than one type of carbapenemases. Those isolates may have a different susceptibility pattern.

In the light of these findings, intravenous fosfomycin administration may be a valuable option in the treatment of CRE infections. For this reason, the determination of fosfomycin susceptibility in CRE strains has become a crucial issue in clinical microbiology laboratories, since the EUCAST recommends the agar dilution method for Enterobacteriaceae, which is not suitable for use in routine laboratory [8].

According to our study results, at least two-third of carbapenemase-positive K. pneumoniae in our center were susceptible to fosfomycin before the availability of the intravenous formulation, and it seems to be used as an alternative adjunct antimicrobial for the treatment of systemic infections caused by carbapenemase-positive K. pneumoniae isolates. It, therefore, seems adequate that laboratories should routinely perform fosfomycin susceptibility testing with reference methods to guide clinicians appropriately.

Compliance with Ethical Standards

Ethical approval: The study protocol was approved by the Marmara University, School of Medicine Ethics Committee (approval number: 09.2018.831).

Conflict of interest: The authors declare that they have no conflict of interest.

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