Biomedical Research 2017; 28 (8): 3731-3735 *ISSN 0970-938X*
www.biomedres.info

Determination of MIC distribution of colistin, fosfomycin, and tigecyclin antibiotics against carbapenem resistant enterobacteriaceae.

Serap Süzük[1*], Banu Kaşkatepe[2], Havva Avcıküçük[3]

[1]Public Health Institution of Turkey Microbiology Reference Laboratories Ankara, Turkey

[2]Department of Pharmaceutical Microbiology, Ankara University Faculty of Pharmacy, Ankara, Turkey

[3]Clinical Microbiology Lab, 29 Mayıs Statement Hospital, Ankara, Turkey

Abstract

Introduction: There are limited treatments options for carbapenem resistant Enterobacteriaceae (CRE) species and especially colistin, fosfomycin and tigecyclin are used for treatment. Therefore, in this study, we aimed for determining the rates of antibiotic susceptibility against colisting, fosfomycin and tigecyclin in CRE isolates with microdilution method.

Material method: In our study, a total of 63 isolates, which were isolated from blood, urine and tracheal aspirate samples. Bacteria identification of the isolates was made with MALDI-TOF MS (Bruker Biotyper; Germany) device. Imipenem, meropenem and ertapenem susceptibilities were determined with the gradient strip test (Liofilchem, Italy) method and genotypic properties were determined with the PCR method. Colistin (0.06-32 μg/ml), fosfomycin (0.06-32 μg/ml) and tigecyclin (0.25-256 μg/ml) MİK values were determined with the microdilution method.

Results: Of the isolates included in the study, 60 isolates (95.24%) were defined as *Klebsiella pneumoniae*, 2 (3.17%) as *Escherichia coli* and 1 (1.59%) as *Enterobacter cloacae*. 57 isolates (90.48%) had OXA-48, 4 (6.35%) had NDM and 2 (3.17%) had OXA-48 and NDM resistant genotypes. MİK50 values for colistin, fosfomycin and tigecyclin were 0.50, 4 and 0.25 MİK90 values were 1, 16 and 1, respectively. Two isolates (3.17%) had resistance to colistin, 23 (63.51%) to fosfomycin and 18 (28.57%) to tigecyclin.

Conclusion: Under the light of the findings of the study, we believe colistin, fosfomycin and tigecyclin can be alternatives for CRE treatment, however, local and national level surveillance studies are important and treatment protocols should be organized and dosage should be arranged based on this surveillance data.

Keywords: Carbapenem resistant enterobacteriaceae, Colistin, Fosfomycin, Tigecyclin, MIC, Microdilution.

Accepted on January 31, 2017

Introduction

Antimicrobial resistance is admitted to be one of the greatest threats to public health. It is a source of concern that the infections caused by enterobacteriaceae-producing carbapenemases are increasingly reported worldwide. They have been known as carbapenem resistant enterobacteriaceae (CRE), resistant to all or most β-lactam antibiotics added last line carbapenems [1]. Carbapenem antibiotics have been used to treat infections reasoned by multi drug resistant gram negative bacteria [2]. On the other hand, high frequency of antibiotic use, misuse of drugs, high rate of healthcare-associated infections, all cause multidrug resistant organisms and these bacteria have been spread out all around the world [3]. The two well-known infectious agents in carbapenem resistant enterobacteriaceae family are carbapenem resistant *Klebsiella pneumonia* and *Escherichia coli* [4].

Production of carbapenemases, which hydrolyze not only carbapenems but also all other beta lactam antibiotics, is mostly responsible for the resistance to carbapenamases. The most five common carbapenemases include imipenemase (IMP) type, veronica integronmetallo beta lactamases (VIM) type, *Klebsiella pneumonia carbapenemase* (KPC) type, New Delhi metallo beta lactamases-1 (NDM-1) and also oxacillinase 48 (OXA 48) which is the most common enzyme in Turkey [5]. Susceptibility to carbapenems is detected by performing phenotypic tests which are enough for antimicrobial susceptibility categorization; nevertheless, it is vital to clarify the detection of resistance mechanism for infection control and public health [6].

Infections caused by CRE have high mortality rates due to very limited available treatment options. High dose carbapenem, polymixin B, colistin, tigecycline, fosfomycin or aminoglyside can be called as preferable CRE treatment options [7]. The

treatment of serious CRE infections should base on Minimal Inhibitor Concentration (MIC) of antibiotic susceptibility results [8]. In this study, we aimed to determine the susceptibility of colistin, tigecycline and fosfomycin in CRE.

Materials and Method

Setting and study design

This is a prospective study that included consecutive inpatients of any age and sex with any infection by any CRE in a period between August 1st 2015-April 30th 2016 in Medical Microbiology Laboratory of 29 Mayıs Statement Hospital, Ankara. In this period, a total of 63 carbapenem resistant enterobacteriaceae species were isolated from blood, urine and tracheal aspirate. Clinical samples were analyzed with standard microbiology laboratory techniques. Only one isolate per patient got involved.

Bacterial identification and phenotypic analysis of carbapenems

All bacterial isolates were identified by MALDI-TOF MS (Bruker Biotyper; Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility tests and extended-spectrum β-lactamases (ESBL) of isolates were analyzed by the BD Phoenix automated system (BD Diagnostics, USA). Minimal inhibitory concentration of ertapenem, imipenem and meropenem were also tested by gradient strip test (Liofilchem, Roseto Degli Abruzzi, Italy). Susceptibility test results of all antibiotics were interpreted according to the EUCAST 2016 break point values [9].

Genotypic methods for detecting carbapanemase gene

A total of 63 Enterobacteriaceae isolates (bla_{OXA-48}, bla_{VIM}, bla_{IMP}, bla_{NDM-1}, bla_{KPC-2} carrying strains) that showed decreased sensitivity to at least one carbapenem (ertapenem, imipenem or meropenem), and carriage of carbapenemase gene confirmed by polymerase chain reaction (PCR), were included in the study [10-14].

Determination of MIC by microbroth dilution

The MICs of colistin, tygecyclin, fosfomycin were determined by the broth micro-dilution method with cation-adjusted Muller-Hinton broth (Oxoid, Code: CM0405, UK) according to Clinical Laboratory Standard Institute (CLSI) guidelines [15-17]. Colistin sulfate (Sigma-Aldrich, St. Louis, MO, USA), tygecycline (Sigma-Aldrich, St. Louis, MO, USA) and fosfomycin (Sigma-Aldrich, St. Louis, MO, USA) were tested over a range of dilutions (0.06-32μg/ml), (0.06-32μg/ml) and (0.25–256 μg/ml) respectively. One hundred microliters of graded concentrations of antibiotics, freshly prepared, were added 96-well U bottom microplates. Bacterial suspensions, which prepared from bacteria grown on non-selective culture media, were added microplates. The microplates were incubated for 24 h at 37°C in ambient air. The breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used for the results as a reference [9].

Escherichia coli ATCC 25922 strain was used as quality control.

Results

Clinical samples for the isolates included in the study and distribution of isolated bacteria are provided in Table 1. 49.21% of the isolates are from urine sample. The most frequently isolated isolate is *K. pneumoniae*. 57 isolates (90.48%) had OXA-48, 4 (6.35%) had NDM and 2 (3.17%) had OXA-48 and NDM resistant genotypes. All of the isolates with NDM as well as OXA-48 and NDM combination were identified in *K. pneumoniae* isolates. MİK50 values for colistin, fosfomycin and tigecyclin were 0.50 μg/ml, 4 μg/ml and 0.25 μg/ml MİK90 values were 1 μg/ml, 16 μg/ml and 1 μg/ml, respectively. Two isolates (3.17%) had resistance to colistin, 23 (63.51%) to fosfomycin and 18 (28.57%) to tigecyclin. It was determined that MİK50 and MİK90 values of the isolates were resistant to all antibiotics (Table 2). Susceptibility of the isolates to antibiotics is provided in Table 3. Both of the colistin resistant isolates were *K. pneumoniae*.

Table 1. *The source and distribution of isolates in clinical samples (n=63).*

Clinical samples	*K. pneumoniae*	*E. coli*	*E. cloace*	**Total**
Tracheal aspirates	5	1	0	6 (9.52)
Blood	24	1	1	26 (41.27)
Urine	31	0	0	31 (49.21)
Total	60 (95.24)	2 (3.17)	1 (1.59)	63 (%100)

Table 2. *Determination and interpretation of the MIC values of the antibiotics tested for CRE (n=63).*

Antibiotic	MIC range	MIC_{50} (μg/ml)	Interpretation*	MIC_{90} (μg/ml)	Interpretation*
Colistin	<0.06-> 32	0.50	Susceptibility	1	Susceptibility
Fosfomycin	< 0.25-> 256	4	Susceptibility	16	Susceptibility
Tigecyclin	<0.06-> 32	0.25	Susceptibility	1	Susceptibility

*The interpretation of the MIC50 and MIC90 of all antibiotics tested for all pathogens was performed using the EUCAST 2016 guidelines.

Table 3. *Determination of the MIC values antibiotics tested for isolates (n=63).*

Bacteria	Colistin		Fosfomycin		Tigecycline	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
K. pneumoniae	58 (96.67)	2 (3.33)	38 (63.33)	22 (36.67)	43 (71.67)	17 (28.33)
E. coli	2 (100.00)	0.00	2 (100.00)	0.00	2 (100.00)	0.00
Enterobacter cloace	1 (100.00)	0.00	0.00	1 (100.00)	0.00	1 (100.00)
Total	61 (96.83)	2 (3.17)	40 (63.49)	23 (36.51)	45 (71.43)	18 (28.57)

Discussion

CRE has been a major public health threat worldwide since the 1990s. CRE infections both cause deterioration of clinical picture and economic losses [18,19]. OXA-48 type carbapenemases that were isolated for the first time in Turkey, are seen commonly in our country. Bacteria with this enzyme have multiple drug resistance, and they have limited the use of antibiotics for the treatment [20]. In this present study, extended spectrumbeta-lactamase positivity was found in 80.95% of isolates along with carbapenemases enzyme positivity and multi drug resistance.

NDM-1 is a type of carbapenemases seen in Saudi Arabia, Kuwait, Amman, United Arab Emirates and Middle East countries [21]. In our country, NDM-1 and NDM-1OXA-48 associations began to be seen in recent years by the reason of developing and spread of medical tourism [22]. We determined 6.35% and 3.17% NDM-1 and NDM-1 and OXA-48 positivity respectively in this present study.

The situation is worrying for CRE infections because of the high mortality rates and treatment options of these infections are limited due to their multi-drug resistance. Therefore, clinicians have included old antibiotics, which are not preferred because of the toxic effects, to the treatment protocols [7]. Colistin, often preferred for treatment of multidrug resistant Gram negative bacteria, has usage restrictions due to its nephrotoxicity [23]. Tigecycline is another antibiotic that is used for CRE infections because of wide tissue distribution. However, FDA defined tigecycline monotherapy as "Boxed Warning" [24]. The other antibiotic that has antimicrobial activity against CRE agents is fosfomycin. Although fosfomycin is often reported as effective in acute uncomplicated urinary tract infection, its intravenous use is preferred for CRE infections [25]. MIC value of agents in CRE infections is important to adjust the dose for treatment protocols. In this study, it was aimed to determine antimicrobial susceptibility of colistin, tigecycline and fosfomycin antibiotics that are preferred for CRE infections, with the micro dilution method.

Colistin, tigecycline and fosfomycin are used as primary treatment options. KPC-producing *K. pneumoniae* treatment algorithms depend on the site of infection. Colistin is the first option especially in blood, lungs and gastrointestinal tract infections and also preferred for urinary tract infections as a possible therapeutic agent. Tigecycline is the first option in the gastrointestinal tract infections and potential therapeutic agents for the blood and lung infections. Fosfomycin, while a first option for urinary tract infections, is among the agents that may be preferred for other sites of infection. MIC levels of antibiotics are important in treatment algorithm because algorithm is determined based on these MIC levels [7].

A total of 96.83% out of 63 CRE isolates, included the study, were found susceptible to colistin, and followed by tigecycline (71.43%) and fosfomycin (63.49%). Colistin was found the most effective antibiotic for our isolates among the tested antibiotics. Only two *K. pneumoniae* isolates were identified as resistant to colistin in all isolates. One *E. cloaca* isolate included in the study was resistant to both fosfomycin and tigecycline. Two *E. coli* strains were found to be sensitive to all antibiotics tested. In a study involving seven Latin American countries, CRA species, isolated from the hospitals of these countries, have been assessed and were found susceptible 96% and 79% of the isolates to colistin and tigecycline respectively [26]. In South Indian region, antibiotic susceptibility of multidrug-resistant *E. coli* and *K. pneumoniae* isolates against various antibiotics were determined by the broth microdilution method. While colistin MIC_{50} and MIC_{90} values of *E. coli* isolates were determined as 0.25 µg/ml and 0.5 µg/ml, for *K. pneumoniae* they were found as 0.5 µg/ml and 1 µg/ml. Also fosfomycin MIC_{50} and MIC_{90} values were found 0.5 µg/ml and 4 µg/ml for *E. coli*, 8 µg/ml and 32 µg/ml for *K. pneumoniae* [27]. Similarly to Indian data, in our study, colistin MIC_{50} and MIC_{90} values were found 0.5 µg/ml and 1 µg/ml, respectively. By contrast, with MIC_{50} and MIC_{90} values differ greatly for fosfomycin.

In 2011, in a study conducted with 81 CRE isolates, antibiotic susceptibilities of colistin, fosfomisin and tigecycline were determined 92.6%, 60.5% and 46.9% respectively. Tigecycline resistant isolates have been reported to be between *K. pneumoniae* and enterobacter spp [28]. Likewise, tigecycline resistant isolates were detected in *K. pneumoniae* and *E. cloace* in our study.

In a surveillance study between 2010-2013 involving hospitals in Europe, it was indicated that CRE isolates were observed to be more often in Poland, Greece and Romania. Also 73.9% and 88.6% of the isolates were reported to be effective with colistin and tigecycline, respectively [29]. In a study evaluating the fecal samples of 100 hospitalized patients colonized with CRE in the intensive care unit, all of the isolates were found susceptible to colistin (MIC range 0,125-1 mg/l) and tigecycline (MIC range 0.06- 1.5 mg/l) [30]. However, the assessment of treatment success in the CRE infection may provide more positive results for the use of these antibiotics. We believe that consideration of the MIC values in the treatments will increase the chance of successful treatment. In our study, we determined that MIC values of isolates approached to the clinical limits. In this case, it should be borne in mind that normal doses of antibiotics may not be the treat levels in plasma.

In accordance with the data obtained from the study, we conclude that colistin, fosfomycin and tigecycline in CRE treatment may be a good option, but for this infection, local and national surveillance studies are important and regulation of treatment protocols and dose adjustment should be done based on surveillance data.

References

1. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med 2012; 18: 263-272.
2. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. Biomed Res Int 2014; 303104.
3. Karuniawati A, Saharman YR, Lestari DC. Detection of carbapenemase encoding genes in Enterobacteriace, Pseudomonasaeruginosa, and Acinetobacterbaumanii isolated from patients at intensive care unit Cipto Mangunkusumo Hospital in 2011. Acta Med Indones 2013; 45: 101-106.
4. Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats in the United States, 2013. Atlanta: CDC.
5. Aktas Z, Kayacan ÇB, Schneider I, Can B, Midilli K, Bauernfeind A. Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in Klebsiellapneumoniae in Istanbul, Turkey. Chemotherapy 2008; 54: 101-106.
6. Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, Malamou-Lada E, Martinez-Martinez L, Navarro F, Nordmann P, Peixe L, Pournaras S, Rossolini GM, Tsakris A, Vatopoulos A, Cantón R. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin Microbiol Infect 2010; 16: 112-122.
7. Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. Treatment options for carbapenem-resistant Enterobacteriaceae infections. Open Forum Infect Dis 2015; 2: ofv050.
8. Lee J, Patel G, Huprikar S, Calfee DP, Jenkins SG. Decreased susceptibility topolymyxin B during treatment for carbapenem-resistant Klebsiella pneumoniae infection. J Clin Microbiol 2009; 47: 1611-1612.
9. European Committee on Antimicrobial Susceptibility Testing. Breakpoints tables for interpretation of MICs and zone diameters.
10. Schechner V, Straus-Robinson K, Schwartz D, Pfeffer I, Tarabeia J, Moskovich R, Chmelnitsky I, Schwaber MJ, Carmeli Y, Navon-Venezia S. Evaluation of PCR-based testing for surveillance of KPC-producing carbapenem-resistant members of the Enterobacteriaceae family. J Clin Microbiol 2009; 47: 3261-3265.
11. Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, Pignatari AC, Tufik S. Rapid detection and identification of metallo-beta-lactamase encoding genes by multiplex real-time PCR assay and melt curve analysis. J Clin Microbiol 2007; 45: 544-547.
12. Biendo M, Canarelli B, Thomas D, Rousseau F, Hamdad F, Adjide C, Laurans G, Eb F. Successive emergence of extended-spectrum beta-lactamase producing and carbapenemase-producing Enterobacter aerogenes isolates in a university hospital. J Clin Microbiol 2008; 46: 1037-1044.
13. Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrob Agents Chemother 2011; 55: 5403-5407.
14. Poirel L, Heritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother 2004; 48: 15-22.
15. Clinical and Laboratory Standards Institute. Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. (9thedn). Wayne (PA): The Institute, 2012.
16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 24th informational supplement. Document M100–S24. Wayne (PA): The Institute, 2014.
17. Humphries RM. Susceptibility testing of the polymyxins: where are we now Pharmacotherapy 2015; 35: 22-27.
18. MacKenzie FM, Forbes KJ, Dorai-John T, Amyes SG, Gould IM. Emergence of a carbapenem-resistant Klebsiella pneumoniae. Lancet 1997; 350: 783.
19. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 2012; 25: 682-707.
20. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48-positive carbapenem-resistant Klebsiella pneumoniae isolates in Istanbul, Turkey. Antimicrob Agents Chemother. 2008; 52: 2950-2954.
21. Sonnevend Á, Ghazawi AA, Hashmey R, Jamal W, Rotimi VO, Shibl AM, Al-Jardani A, Al-Abri SS, Tariq WUZ, Weber S, Pál T. Characterization of carbapenem-resistant

Enterobacteriaceae with high rate of autochthonous transmission in the arabian peninsula. PLoS One 2015 ;10: e0131372.

22. Karabay O, Altindis M, Koroglu M, Karatuna O, Aydemir ÖA, Erdem AF. The carbapenem-resistant Enterobacteriaceae threat is growing: NDM-1 epidemic at a training hospital in Turkey. Ann Clin Microbiol Antimicrob 2016; 15: 16-22.
23. Gales AC, Jones RN, Sader HS. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006–09). J Antimicrob Chemother 2011; 66: 2070-2074.
24. Dixit D, Madduri RP, Sharma R. The role of tigecycline in the treatment of infections in light of the new black box warning. Expert Rev Anti Infect Ther 2014; 12: 397-400.
25. Reffert JL, Smith WJ. Fosfomycin for the treatment of resistant gram-negative bacterial infections. Insights from the Society of Infectious Diseases. Pharmacists.Pharmacotherapy 2014; 34: 845-857.
26. Villegas MV, Pallares CJ, Escandón-Vargas K, Hernández-Gómez C, Correa A, Álvarez C, Rosso F, Matta L, Luna C, Zurita J, Mejía-Villatoro C, Rodríguez-Noriega E, Seas C, Cortesía M, Guzmán-Suárez A, Guzmán-Blanco M. Characterization and Clinical Impact of Bloodstream Infection Caused by Carbapenemase-Producing Enterobacteriaceae in Seven Latin American Countries. PLoS One 2016; 11: e0154092.
27. Rajenderan S, Balaji V, Anandan S1, Sahni RD, Tansarli GS, Falagas ME. Determination of MIC distribution of arbekacin, cefminox, fosfomycin, biapenem and other antibiotics against gram-negative clinical isolates in South India: a prospective study. PLoS One 2014; 9: e103253.
28. Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. Int J Antimicrob Agents 2011; 37: 415-419.
29. Sader HS, Castanheira M, Flamm RK, Mendes RE, Farrell DJ, Jones RN. Tigecycline activity tested against carbapenem-resistant Enterobacteriaceae from 18 European nations: results from the SENTRY surveillance program (2010-2013). Diagn Microbiol Infect Dis 2015; 83: 183-186.
30. Mittal G, Gaind R, Kumar D, Kaushik G, Gupta KB, Verma PK, Deb M. Risk factors for fecal carriage of carbapenemase producing Enterobacteriaceae among intensive care unit patients from a tertiary care center in India. BMC Microbiol 2016; 16:138-148.

*Correspondence to

Serap Süzük

Public Health Institution of Turkey Microbiology Reference Laboratories

Adnan Saygun Caddesi,

Sıhhiye,

Ankara,

Turkey