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ORIGINAL ARTICLE

Curcumin-meropenem synergy in carbapenem resistant *Klebsiella pneumoniae* curcumin-meropenem synergy

Dumrul Gülen, Birol Şafak*, Berna Erdal, Betül Günaydın

Department of Medical Microbiology, Tekirdağ Namık Kemal University, Faculty of Medicine, Tekirdağ, Turkey

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ABSTRACT

Background and Objectives: The frequency of multiple resistant bacterial infections, including carbapenems, is increasing worldwide. As the decrease in treatment options causes difficulties in treatment, interest in new antimicrobials is increasing. One of the promising natural ingredients is curcumin. It is known to be effective in bacteria such as *Pseudomonas aeruginosa, Escherichia coli, Burkholderia pseudomallei* through efflux pump inhibition, toxin inhibition and enzymes. However, because its bioavailability is poor, it seffectiveness occurs in combination with antibiotics. In the study, the interaction of meropenem and curcumin in carbapenemase producing strains of *Klebsiella pneumoniae* was tested.

Materials and Methods: Thirty-nine *Klebsiella pneumoniae* isolates, resistant to meropenem, were used in this study. From those 15 MBL, 6 KPC, 17 OXA-48 and 1 AmpC resistance pattern were detected by combination disk method. Meropenem and Curcumin MIC values were determined by liquid microdilution. Checkerboard liquid microdilution was used to determine the synergy between meropenem and curcumin.

Results: Synergistic effects were observed in 4 isolates producing MBL, 3 isolates producing KPC, 4 isolates producing OXA-48, and 1 isolates producing AmpC (totally 12 isolates) according to the calculated FICI. No antagonistic effects were observed in any isolates.

Conclusion: Curcumin was thought to be an alternative antimicrobial in combination therapies that would positively contribute to the treatment of bacterial infection. The effectiveness of this combination should be confirmed by other *in vitro* and clinical studies.

Keywords: Curcumin; *Klebsiella pneumoniae*; Carbapenemase; Drug synergism; Anti-bacterial agents

INTRODUCTION

The Enterobacteriaceae family has a broad clinical picture ranging from simple community acquired infections to hospital acquired infections (1). Increased antimicrobial drug resistance, especially in infections associated with medical care, leads to increased morbidity and mortality (1-3). In recent years, the frequency of multiple drug resistance isolates including carbapenems has been increasing in the world (2). The development of multidrug resistance leads to treatment failure.

The production of various carbapenemase enzymes like beta lactamase are responsible for the majority of carbapenem resistance in enteric bacteria. These enzymes include class A *Klebsiella pneumoniae* carbapenemase (KPC), class B metallo-beta-lactamases (MBL) and class D oxacillinases (OXA-48-like) (4). Carbapenemase containing isolates are general-

*Corresponding author: Birol Şafak, MD, Department of Medical Microbiology, Tekirdağ Namık Kemal University, Faculty of Medicine, Tekirdağ, Turkey.
Tel: +90-2822505500
Fax: +90-2822509928
Email: bsafak@nku.edu.tr

ly resistant to fluoroquinolone, aminoglycoside, and co-trimoxazole, as well as becoming resistant to all beta-lactam antibiotics. By plasmid mediated transition, carbapenemases spread rapidly around the world. Globalization, refugee flows, pilgrimage and health tourism have significantly contributed to this spread. KPC was firstly isolated in the US, then also reported in Europe and China. Imipenemases (IMP-1) is more common in Japan, while New Delhi metallo-β lactamase (NDM-1) is more common in Pakistan and India. In many European countries, isolates containing OXA-48 cause outbreaks with rapidly increasing prevalence (4-7).

Due to its increasing frequency, carbapenemase-producing *Enterobacteriaceae* (CPE) are monitored by forming surveillance networks. A 2015 report by the European Centre for Disease Prevention and Control (ECDC) highlights an increasing frequency of CPE between 2010 and 2015 (1). Additionally, when the reports published by the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) network of the World Health Organization (WHO) in 2016 and 2017 are observed, it is seen that the frequency is increasing by years (3, 8). In addition to active surveillance, comprehensive measures such as hand hygiene, early diagnosis, contact measures and isolation, environmental cleaning and antibiotic management are used to combat CPE (9).

The multiple resistance pattern of antibiotics used in treatment increases the interest in newly developed antimicrobials. Natural ingredients, which have been used in traditional medicine in many countries for centuries, are the active substances of modern medical drugs (Atropa belladonna, Salixalba, Digitalis purpurea) and show promise in this regard. One of these components is curcumin, which is the main component of *Curcuma longa* L. belonging to the Zingiberaceae family extracted from the rhizomes (10). In addition to supplement, spice and food additive, it is also used for medical purposes all over the world (11). Curcumin has several molecular targets such as various transcriptional factors, inflammatory cytokines, enzymes, kinases, growth factors, receptors, adhesion molecules, and antiapoptotic proteins in the tissue (12). Anti-inflammatory, antioxidant, anti-venom, anti-HIV, anti-tumor, anti-apoptotic, burn wound healing, antiprotozoal, nematocidal, anti-retroviral, antifungal, antimalarial and antibacterial effects have been shown (10, 13-17).

It has been shown in previous studies that curcumin exhibited antibacterial activity: FtsZ protein inhibition involved in prokaryotic cell division; efflux pump inhibition in Gram negative bacteria; inhibition of PAO1 virulence factors in *Pseudomonas aeruginosa*; inhibition of Pet and EspC toxin secretion in Enteroagregative *Escherichia coli* and Enteropathogenic *E. coli* strains; inhibition of lipase, protease and biofilm formation in *Burkholderia pseudomallei* (18-22). However, poor bioavailability and low plasma concentration reduce its effectiveness. Antimicrobial activity occurs in combination with antibiotics (23). When combined with antimicrobials, synergy has been observed with some, while antagonism has been observed with others (24, 25).

In this study, we aimed to demonstrate the interaction of meropenem and curcumin in carbapenemase producing *K. pneumoniae* strains and its relationship with phenotypic resistance pattern.

MATERIALS AND METHODS

Bacterial strains. Thirty nine meropenem resistant *K. pneumoniae* isolates cultured from various clinical specimens at Tekirdağ Namık Kemal University Medical Microbiology Laboratory during January 2018 and January 2019 were included in the study. Meropenem resistance was determined according to the recommendations of The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Accordingly, those with meropenem MIC value >0.125, meropenem inhibition zone diameter <25mm, or meropenem inhibition zone diameter 25-27 mm of piperacillin resistant isolates were considered meropenem resistant. All isolates were stored in Mueller-Hinton broth containing 15% glycerol at -80°C until tested. Before the study, all isolates were passaged to 5% sheep blood agar.

Determination of carbapenemase phenotype. For the determination of carbapenem resistance mechanism, MASTDISCS® CombiCarba plus *(Enterobacteriaceae)* disc system (Mast Group, Merseyside, UK) was used. It was prepared at a density of 0.5 McFarland suspension from a fresh blood agar passage made of the strain to be tested. Using the Kirby-Bauer disk diffusion test principles, Penem disc 10 µg, Penem 10 µg + MßL inhibitor disc, Penem 10 µg + KPC inhibitor disc, Penem 10 µg + AmpC inhibitor disc

and Temosilin + MßL inhibitor disc were placed on Mueller Hinton Agar (MHA) plate. After 16-24 hours of incubation at 36°C ± 1°C, the diameters of the disc zones were interpreted. *K. pneumoniae* NCTC 13438, *K. pneumoniae* NCTC 13440, *K. pneumoniae* NCTC 13442 were used as control strain.

Chemical reagents and media. Curcumin compound (67% purity) and meropenem (MEM) (≥98% purity) were obtained from Sigma Chemicals Co. (St Louis, Missouri, USA). They were stored at -20°C until extraction as a powder and allowed to warm to room temperature before the experiment.

Determination of minimum inhibitory concentrations. For all strains, minimum inhibitor concentrations (MIC) were calculated by microdilution method for meropenem and curcumin. Meropenem and curcumin as the active ingredient were suspended in accordance with the manufacturer's recommendations, and stock solutions were prepared. Serial dilutions were made in Mueller Hinton broth on microdilution plates. The dilution ranges were 0.5-256 mg/L for meropenem and 2-1024 mg/L for curcumin. After the suspension was prepared in 0.5 McFarland (10^8 cfu/ml) standard turbidity of all isolates, the final bacterial concentration was inoculated to microdilution plates to be 5×10^5 cfu/ml, and the microplates were incubated at 36°C ± 1°C for 18-20 hours. The lowest concentration without bacterial growth was determined as the MIC value. The determined MIC values were classified according to phenotypic resistance patterns.

Determination of *in vitro* synergy of meropenem-curcumin combinations. In the strains determined as meropenem-resistant and moderately sensitive using EUCAST criteria, the checkerboard microdilution method was used to evaluate the synergy between meropenem and curcumin. For this test, 50 µl Mueller Hinton broth was distributed to all wells of 96 well microplate, and serial dilutions of meropenem (0.5-256 mg/L) on the horizontal axis and curcumin (8-1024 mg/L) on the vertical axis were made. Bacterial suspensions of 0.5 McFarland (10^8 cfu/ml) standard turbidity from all isolates were prepared, diluted 1:10, and inoculated as 5 µl in each well of 100 µl volume to ensure that the final bacterial concentration in each well was 5×10^5 cfu/ml. Microplates were incubated for 18-20 hours at 37°C. Synergy relationship was evaluated by calculating Fractional Inhibitor Concentration Index (FICΣ). The synergy between curcumin and Meropenem was determined by calculating the FICΣ as described previously.

$$FIC\Sigma = \frac{\text{MIC value of Curcumin in combination}}{\text{MIC value of Curcumin alone}} + \frac{\text{MIC value of Meropenem in combination}}{\text{MIC value of Meropenem alone}}$$

It was interpreted as synergy if the FICΣ value was ≤0.5, additive effect if >0.5-1, indifference effect if >1-4, and antagonist effect if >4 (26).

RESULTS

Fifteen out of the 39 isolates studied had MBL resistance pattern, 6 had KPC resistance pattern, 17 had OXA-48 resistance pattern, and 1 had AmpC resistance pattern. The MIC values measured alone were between 4-256 mg/L for meropenem and 1024 mg/L for curcumin in all isolates. MIC values in the combination ranged between 0.5-256 mg/L for meropenem and 8-1024 mg/L for curcumin. MIC values and FICΣ of isolates alone and in combination are shown in Table 1. According to calculated FICΣs, synergistic effect was observed in a total of 12 isolates as 4 isolates producing MBL, 3 isolates producing KPC, 4 isolates producing OXA-48 and 1 isolate producing AmpC. No antagonistic effect was observed in any isolate. Synergistic effect interpretations according to phenotypic resistance patterns are shown in Table 2.

DISCUSSION

The dramatic increase in the clinical effect and prevalence of infections caused by carbapenemase producing bacteria, especially in the *Enterobacteriaceae* family, is a global health problem. Because it is easily spread and colonized in health care circles, preventing transition is a major public health problem (2).

EUCAST recommends the use of meropenem for carbapenemase screening. Combination disc test, colorimetric tests, carbapenem inactivation method, MALDI-TOF and phenotypic methods such as lateral flow are recommended for screening (27). Considering these criteria, carbapenemase resistance

Table 1. MIC values and FICΣ of isolates alone and in combination

Isolates	Isolates Number	MICs in monotherapy (mg/L)		MICs in combination (mg/L)		FICΣs	Comments
		MEM	C	MEM	C		
MBL isolates	AB 40	256	1024	0,5	1024	1	Additive
	AB 56	16	1024	0,5	8	0,03	Synergy
	AB 331	128	1024	0,5	8	0,01	Synergy
	AB 610	16	1024	0,5	8	0,03	Synergy
	AB 1752	256	1024	0,5	1024	1	Additive
	AB 2697	256	1024	0,5	8	0,009	Synergy
	AB 3380	16	1024	32	8	2	Indifference
	AB 3722	16	1024	64	8	4	Indifference
	AB 4628	256	1024	0,5	1024	1	Additive
	AB 4784	32	1024	128	8	4	Indifference
	AB 4945	64	1024	128	8	2	Indifference
	AB 793	32	1024	128	8	4	Indifference
	AB 797	64	1024	128	8	2	Indifference
	AB 820	8	1024	0,5	1024	1	Additive
	AB 1	128	1024	256	8	2	Indifference
KPC isolates	AB 98	16	1024	0,5	8	0,03	Synergy
	AB 256	16	1024	0,5	8	0,03	Synergy
	AB 530	16	1024	32	8	2	Indifference
	AB 1394	16	1024	32	8	2	Indifference
	AB 2557	256	1024	0,5	8	0,009	Synergy
	AB 4568	32	1024	32	8	1	Additive
OXA-48 isolates	AB 290	16	1024	1	8	0,07	Synergy
	AB 1105	16	1024	64	8	4	Indifference
	AB 1380	8	1024	2	8	0,25	Synergy
	AB 2043	4	1024	4	8	1	Additive
	AB 2509	4	1024	4	8	1	Additive
	AB 3235	8	1024	0,5	1024	1	Additive
	AB 3322	16	1024	0,5	8	0,03	Synergy
	AB 4835	128	1024	0,5	512	0,5	Synergy
	AB 2	128	1024	256	8	2	Indifference
	AB 4	16	1024	32	8	2	Indifference
	AB 5	16	1024	32	8	2	Indifference
	AB 7	32	1024	64	8	2	Indifference
	AB 9	32	1024	64	8	2	Indifference
	AB 12	16	1024	64	8	4	Indifference
	AB 13	16	1024	32	8	2	Indifference
	AB 14	16	1024	32	8	2	Indifference
	AB 15	32	1024	32	8	1	Indifference
AmpC isolates	AB 374	64	1024	0,5	8	0,01	Synergy

C, Curcumin; KPC, *Klebsiella pneumoniae* carbapenemase; MBL, metallo-beta-lactamases; MEM, meropenem

was sought in meropenem resistant *K. pneumoniae* isolates with a combination disc test with reported sensitivity between 82.3-100% and specificity between 97.1-100% and recommended for use in the basic microbiology laboratory (28). Among the isolates studied, the highest rate of resistance mecha-

Table 2. Synergistic effect interpretations according to phenotypic resistance patterns

Resistance phenotype	Synergistic effect		Additive effect		Antagonistic effect	
	n	%	n	%	n	%
MBL (15)	4	26,7	11	73,3	0	0
KPC (6)	3	50	3	50	0	0
OXA-48 (17)	4	23,5	13	76,5	0	0
AmpC (1)	1	100	0	0	0	0

KPC, *Klebsiella pneumoniae* carbapenemase; MBL, metallo-beta-lactamases

nism was found to be OXA-48 in accordance with Turkish Data (1, 4).

Combination therapies play an important role in the treatment of *K. pneumoniae* strains producing carbapenemase. It was emphasized that there was no significant difference in mortality rates between monotherapy and untreated patients. It has been reported that combination therapy reduces mortality and that the lowest mortality rates are seen in combinations containing carbapenem (29).

For this purpose, combination susceptibility tests of carbapenems and colistin, tigecycline, sulbactam, fosfomycin, aminoglycosides were examined and various synergy levels were obtained (30-32). The fact that these combination therapies have varying levels of synergy has led to the search for new treatment alternatives, and numerous studies have been reported on antibiotic-natural compound combination synergy for this purpose (33).

It has been reported that natural products with low molecular weight increase the effect of antifungal and antibacterial agents (23). Essential oil components and derivatives have been used in combination with antibiotics. One of them has shown the synergistic effect of antibiotic therapy combined with curcumin in the treatment of methicillin resistant S. *aureus* (24).

In one study, the synergistic effect of curcumin with antibiotics has been reported to prevent biofilm formation (34). In another study, antagonistic effects were reported in the use of ciprofloxacin with curcumin for *Salmonella enterica* Serovar Typhimirium and *Salmonella enterica* Serovar Typhi (25). In our study, 30.7% synergy was observed between curcumin and meropenem against carbapenem resistant *K. pneumoniae* isolates, whereas no strain showed antagonistic effect. Limited studies have been reported on synergistic activity to carbapenem resistance mechanisms. In these studies, higher synergistic effects have been observed in several combinations of NDM containing *K. pneumoniae* isolates (31). In our study, higher curcumin-meropenem synergy was observed in *K. pneumoniae* isolates producing KPC. The optimum potential of curcumin is limited due to its poor oral bioavailability, poor absorption, rapid metabolism and rapid systemic elimination and inadequate dissolution in aqueous solvents. Numerous studies are conducted to increase the bioavailability of curcumin (17). Due to the lack of effective antibiotics against *K. pneumoniae*, which produces carbapenemase, combination therapy appears to be a useful strategy to provide clinical efficacy and prevent the development of resistance.

CONCLUSION

We did not detect any antagonism between curcumin and meropenem against *K. pneumoniae*, which produces carbapenemase, and we detected higher synergies especially in isolates containing KPC. We think that curcumin may an ideal nutritional supplement that will positively affect the process and even a good antimicrobial alternative in the treatment of many bacterial infections, including resistant strains. Further *in vitro* and clinical studies are needed to determine of bioavailability and confirm the effectiveness of this combination of drugs.

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