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IN VITRO ACTIVITY OF CEFTAZIDIME-AVIBACTAM AND COLISTIN AGAINST CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

SEFTAZİDİM-AVİBAKTAM VE KOLİSTİNİN KARBAPENEM DİRENÇLİ *PSEUDOMONAS AERUGİNOSA* KLİNİK İZOLATLARINA KARŞI İN VİTRO AKTİVİTESİ

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ABSTRACT

Objective: Infections caused by multi drug-resistant Gram-negative bacilli are increasingly reported worldwide. Ceftazidime-avibactam is a novel antibiotic combination that presents good activity against carbapenem-resistant *Enterobacterales* members and *Pseudomonas aeruginosa* isolates. The objective of this study was to evaluate the in vitro activity of ceftazidime-avibactam and colistin against carbapenem-resistant *P. aeruginosa* isolates.

Materials and Methods: A total of 100 carbapenem-resistant and non-duplicate *P. aeruginosa* isolates obtained from patient samples in our hospital between 2016-2021 were included in the study. The isolates were identified by MALDI-TOF MS (Bruker Daltonics, Germany). The minimum inhibitory concentration (MIC) values of meropenem, colistin, ceftazidime, and ceftazidime-avibactam were determined by the broth microdilution method. The presence of carbapenemase genes *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{OXA-48}$, *bla* $_{IMP}$, and *bla* $_{VIM}$ were investigated using PCR.

Results: Carbapenemase genes were not detected among the isolates except for one isolate producing *bla* $_{VIM}$. The susceptibility rates of ceftazidime-avibactam and colistin were 90% (n=90) and 100% (n=100), respectively. The MIC_{50} and MIC_{90} values for meropenem, ceftazidime, ceftazidime-avibactam, and colistin against *P. aeruginosa* isolates were found to be 32/64, 8/64, 4/8, 0.5/2 µg/mL, respectively.

ÖZET

Amaç: Çok ilaca dirençli Gram negatif basillerin neden olduğu enfeksiyonlar dünya genelinde giderek daha fazla rapor edilmektedir. Seftazidim-avibaktam, karbapenem dirençli *Enterobacterales* üyelerine ve *Pseudomonas aeruginosa* izolatlarına karşı iyi etkinlik gösteren yeni bir antibiyotik kombinasyonudur. Bu çalışmanın amacı, seftazidim-avibaktam ve kolistinin karbapenem dirençli *P. aeruginosa* izolatlarına karşı in vitro aktivitesini değerlendirmektir.

Gereç ve Yöntem: Hastanemizde 2016-2021 yılları arasında hasta örneklerinden elde edilen toplam 100 karbapenem dirençli ve tekrar içermeyen *P. aeruginosa* izolatı çalışmaya dahil edildi. İzolatlar MALDI-TOF MS (Bruker Daltonics, Almanya) ile tanımlandı. Meropenem, kolistin, seftazidim ve seftazidim-avibaktamın minimum inhibitör konsantrasyon (MİK) değerleri sıvı mikrodilüsyon yöntemi ile belirlendi. Karbapenemaz genlerinden *bla* $_{KPC}$, *bla* $_{NDM}$, *bla* $_{OXA-48}$, *bla* $_{IMP}$, ve *bla* $_{VIM}$ varlığı PCR ile araştırıldı.

Bulgular: Bir *bla* $_{VIM}$ üreten izolat dışında izolatlarda karbapenemaz genleri saptanmadı. Seftazidim-avibaktam ve kolistinin duyarlılık oranları sırasıyla %90 (n=90) ve %100 (n=100) bulundu. *P. aeruginosa* izolatlarına karşı meropenem, seftazidim, seftazidim-avibaktam ve kolistin için $MİK_{50}$ ve $MİK_{90}$ değerleri sırasıyla 32/64, 8/64, 4/8, 0,5/2 µg/mL olarak bulundu.

Sonuç: Verilerimiz seftazidim-avibaktamın karbapenem dirençli *P. aeruginosa* izolatlarının tedavisi için iyi bir alternatif seçenek

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Conclusion: The data suggests that ceftazidime-avibactam exhibits as a viable alternative for the treatment of carbapenem-resistant *P. aeruginosa* isolates. It's noteworthy that colistin resistance was not detected among the study isolates. Rational use of antibiotics should be emphasized to prevent the development of antibiotic resistance. Surveillance of ceftazidime-avibactam and colistin should be followed up with routine antimicrobial susceptibility tests.

Keywords: Ceftazidime-avibactam, colistin, carbapenem, *Pseudomonas aeruginosa*

olduğunu göstermektedir. Çalışma izolatları arasında kolistin direnci tespit edilmemesi dikkat çekicidir. Antibiyotiklere direnç gelişiminin önlenmesi için akılcı antibiyotik kullanımına önem verilmelidir. Seftazidim-avibaktam ve kolistin sürveyansı rutin antimikrobiyal duyarlılık testleri ile takip edilmelidir.

Anahtar Kelimeler: Seftazidim-avibaktam, kolistin, karbapenem, *Pseudomonas aeruginosa*

INTRODUCTION

The World Health Organization (WHO) specified primacy pathogens for the research and development of new antibiotics in 2017. Carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and members of *Enterobacteriaceae* were indicated as critical pathogens (1). *P. aeruginosa*, a non-fermentative Gram-negative rod, is a significant pathogen causing antibiotic-resistant nosocomial infections such as bacteremia, pneumonia, skin and soft tissue infections, and urinary tract infections. In addition to nosocomial infections, serious community-acquired bacterial infections caused by *P. aeruginosa* were also encountered (2, 3). It has become a factor in increasing mortality and morbidity rates due to infections and has triggered the development of resistance to antibiotics in many countries, including Turkiye. Carbapenems are mainly preferred in the treatment of *P. aeruginosa*-related systemic infections resistant to beta-lactam/beta-lactamase inhibitor antibiotics and cephalosporins. However, in cases of resistance to carbapenems, there are limited treatment options for which polymyxins are the mainstay of therapy. Other treatment options include aminoglycosides and tigecycline (4). Carbapenem resistance in *Pseudomonas* species may develop through the combinations of carbapenemases or metallo-beta-lactamases (MBLs), Ambler class A or B beta-lactamases, *Amp*C production, efflux pump upregulation, and *opr*D porin mutations Carbapenems are stable to *Amp*C cephalosporinases alone, but activity may be attenuated by combinations of Ambler class A or B ß-lactamases, *Amp*C production, efflux pump upregulation, and *opr*D porin mutations (5). Avibactam is a non-beta-lactam-beta-lactamase inhibitor that acts on class A beta-lactamases, including extended spectrum beta-lactamases (ESBLs) and *Klebsiella pneumoniae* carbapenemases (KPC), Ambler class C beta-lactamases, and some class D beta-lactamases (4, 5).

There is an urgent need for novel antibiotics effective against these multi- and pan-resistant pathogens called superbugs. Ceftazidime-avibactam is a novel antibiotic combination permitted by the United States Food and Drug Administration (FDA) in 2015 for use in complex intra-abdominal infections, complicated urinary tract infections, nosocomial and ventilator-associated pneumonia, and has good efficacy against carbapenem-resistant *Enterobacterales,* and *Pseudomonas* spp. (6). Polymyxins with hydrophilic and lipophilic properties, discovered in 1947, have a bactericidal effect against Gram-negative bacteria with a detergent-like effect. While polymyxins are classified into five groups as A, B, C, D, and E to the chemical components, only polymyxin B and polymyxin E are in clinical use. Colistin (Polymyxin E), a member of polymyxin group antibiotics, has been used as a last-line antibiotic option for carbapenem-resistant Gram-negative bacilli infections. It binds to phosphate groups of lipid A, a key component of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, via electrostatic interaction and plays a key role in cell permeability and extracellular exchange. Colistin effects the divalent cations (Ca^{2+} and Mg^{2+}) from the phosphate groups of membrane lipids. As a result, the outer membrane is destroyed, the cell contents are discharged, and the bactericidal effect is observed (7, 8). In addition to the current problems of limited efficacy and toxicity, increasing resistance to colistin is emerging in some areas. The efficacy of ceftazidime-avibactam and colistin against multidrug-resistant isolates (MDR), including those resistant to carbapenems, has been investigated in several studies. Antibiotic susceptibility may vary depending on geographic and institutional conditions and the resistance pattern of isolates in the study population. The purpose of the present study was to evaluate in vitro activity of ceftazidime-avibactam and colistin against carbapenem-resistant *P. aeruginosa* isolates and to establish five years of antimicrobial surveillance data on MDR *P. aeruginosa* isolates in our institution.

MATERIALS AND METHODS

Bacterial isolates

One hundred carbapenem-resistant *P. aeruginosa* isolates collected from clinical samples of patients at a tertiary training and research hospital in Turkiye from January 1, 2016, to January 31, 2021, were evaluated in the study. Duplicate isolates were excluded from the study. The isolates were collected from blood (n=9), endotracheal aspirate (n=12), wound (n=22), transtracheal aspi-

rate (n=17), tissue/pus (n=14), sputum (n=10), urine (n=9), pleural fluid (n=5), bile acid (n=1), and cerebrospinal fluid (n=1). Matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Brucker Daltonics, Bremen, Germany) was used for identification of isolates. The strains were stored in the brain-heart infusion broth supplemented with 15% glycerol. As a final step, all isolates were stored at -20°C for further steps. This study was approved by the Ethical Committee of the University of Health Sciences (Date: 22.10.2019, No: 19/320).

Antimicrobial susceptibility testing (AST)

In routine laboratory tests, susceptibility of isolates to carbapenems and other antibiotics was performed by the Kirby-Bauer disk diffusion method and/or VITEK2 system (bio-Merieux, France). The susceptibility of isolates to meropenem, colistin, ceftazidime, and ceftazidime-avibactam was assessed by broth microdilution as recommended by the Clinical and Laboratory Standards Institute (CLSI) (9). Antimicrobial stock solutions were prepared according to the manufacturers' recommendations and stored frozen at -80°C until used in susceptibility tests. In the broth microdilution method, serial two-fold dilutions ranging from 128–0.125 µg/mL for ceftazidime and ceftazidime-avibactam, from 512–0.5 µg/mL for meropenem, and from 16–0. 5 µg/mL for colistin were prepared in 96-well microtiter plates containing fresh cation-adjusted Mueller Hinton Broth. Avibactam was combined with ceftazidime, fixed at a concentration of 4 µg/mL. The minimum inhibitor concentration (MIC) values of isolates were assessed as suggested by the European Committee of Antimicrobial Susceptibility Testing (EUCAST) (10). *P. aeruginosa* ATCC® 27853, *E. coli* ATCC® 25922, *K. pneumoniae* ATCC® 700603 and *E. coli* NCTC 13846 (*mcr*-1 positive) were used as controls in AST.

DNA extraction for polymerase chain reaction (PCR)

DNA extraction was accomplished using the boiling method as described in a previous study (11). PCR assays were performed using a thermal cycler (T100™, Bio-Rad, USA). The presence of carbapenemase genes (*bla* $_{OXA-48}$, *bla* $_{NDM}$, *bla* $_{KPC}$, *bla* $_{IMP}$, *bla* $_{VIM}$) were investigated by singleplex PCR using specific primers presented in Table 1 (12-14). Positive control isolates for evaluated genes were used in all tests. PCR amplifications for carbapenem resistance genes were performed in 25-µL PCR mixture containing 2.5 µL of total DNA, 1X PCR buffer, 2.5 mM of $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 20 pmol of each primer, and 0.25 U of Taq DNA polymerase (5 U/µl, ABM, Canada) except *bla* $_{KPC}$. A total of 25 µL PCR mixture including 2.0 µL of bacterial DNA, 1X PCR buffer, 2.0 mM of $MgCl_2$, 0.25 mM of each dNTPs, 20 pmol of each primer, and 1 U of Taq DNA polymerase (5 U/µl, ABM, Canada) was used for *bla* $_{KPC}$. The amplification programs for *bla* $_{NDM}$ *and bla* $_{OXA-48}$, *bla* $_{VIM}$ and *bla* $_{IMP}$, *bla* $_{KPC}$ were used as indicated in previous studies. PCR products were analyzed after electrophoresis at 100 V for 40 minutes on a 1% agarose stained with safe dye (Safe View, ABM, Canada). PCR fragments were visualized under UV light based on fragment size.

RESULTS

Of the one hundred *P. aeruginosa* isolates, fifty-one were obtained from patients in intensive care units, and forty-nine were obtained from hospitalized patients. Ninety (90%) *P. aeruginosa* isolates consisted of blood, endotracheal aspirate, wound swab, transtracheal aspirate, tissue, sputum, and urine culture samples. Of the evaluated carbapenemase genes, only *bla* $_{VIM}$ was detected in one *P. aeruginosa* isolate (Figure 1). According to the meropenem susceptibility test results detailed in EUCAST criteria, eleven (11%) *P. aeruginosa* isolates were categorized as

Table 1: Primers used in the study

Beta lactamase genes	Primer sequences (5'-3')	PCR products (bp)	References
bla $_{OXA-48}$	GCGTGGTTAAGGATGAACAC	438	12
	CATCAAGTTCAACCCAACCG		
bla $_{NDM}$	GCAGCTTGTCGGCCATGCGGGC	782	12
	GGTCGCGAAGCTGAGCACCGCAT		
bla $_{VIM}$	GATGGTGTTTGGTCGCATA	390	13
	CGAATGCGCAGCACCAG		
bla $_{IMP}$	GGAATAGAGTGGCTTAAYTCT	188	13
	CCAAACYACTASGTTATCT		
bla $_{KPC}$	TGTCACTGTATCGCGGTC	900	14
	CTCAGTGCTCTACAGAAAAAC		

Bp: Base pair

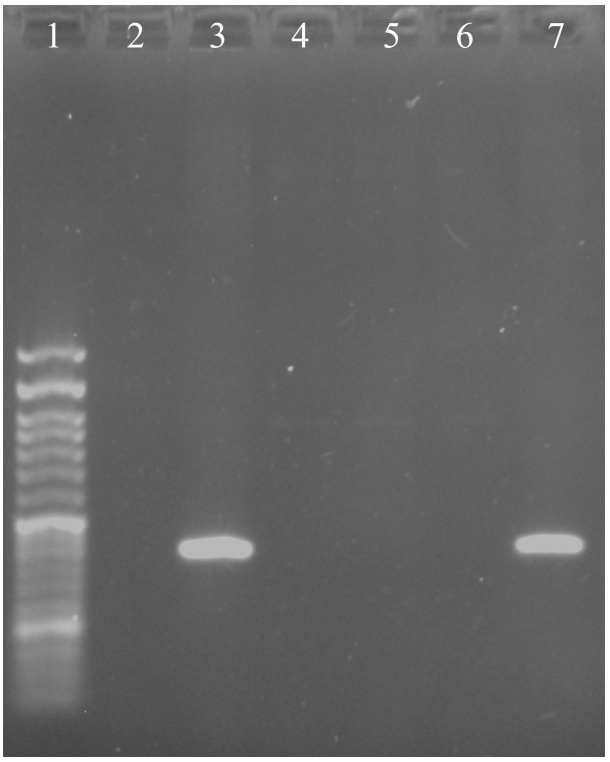

Figure 1: Agarose gel image of the *bla* $_{VIM}$ gene amplified by singleplex PCR

Well 1: DNA ladder, well 2: negative control, well 3: positive control (*bla* $_{VIM}$-390 bp), well 4-6: negative strains, well 7: *bla* $_{VIM}$ positive strain

"susceptible, increased exposure". Overall, the susceptibility rates for ceftazidime-avibactam and colistin were 90% (n=90) and 100% (n=100) among *P. aeruginosa* isolates, respectively. The MIC_{50} and MIC_{90} values for meropenem, ceftazidime, ceftazidime-avibactam, and colistin against *P. aeruginosa* isolates were determined to be 32/64, 8/64, 4/8, 0.5/2 µg/mL, respectively. The antimicrobial susceptibility of isolates detected by broth microdilution is presented in Table 2. The susceptibility of the isolates to antibiotics accomplished in routine tests are represented in Table 3.

DISCUSSION

P. aeruginosa is one of the major causes of serious nosocomial infections such as bacteremia, pneumoniae, urinary tract infections, wound, and burn site infections. The antimicrobial resistance is emerging even for last choice antibiotics among these bacteria. The prevalence of MDR isolates has been increasing worldwide in recent years and the treatment options are extremely limited. Beside the intrinsic antibiotic resistance, the ability to develop acquired resistance mechanisms such as modification of drug or target sites, activated expression of efflux pumps, alteration of cell wall permeability, acquisition of resistance genes, enzymatic inactivation challenge the treatment of *P. aeruginosa*-associated infections and increase the mortality rates and healthcare costs (15, 16). Agents such as colistin, aminoglycosides, and tigecycline remain the last choice of drugs for MDR isolates. A few

Table 2: Antimicrobial susceptibility of *P. aeruginosa* isolates by broth microdilution (n=100)

Antibiotics	MIC ranges (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	S (SDR) n (%)	S (IE) n (%)	R n (%)
Meropenem	512-0.5	32	64	-	11 (11)	89 (89)
Ceftazidim	128-0.125	8	64	-	57 (57)	43 (43)
Ceftazidime-avibactam	128-0.125	4	8	90 (90)	-	10 (10)
Colistin	16-0.250	0.5	2	100 (100)	-	-

MIC: Minimum Inhibitory Concentration, S (SDR): Susceptible, standard dosing regimen, S (IE) : Susceptible, increased exposure, R: Resistant

Table 3: Susceptibility of *P. aeruginosa* isolates to other antibiotics

Antibiotics	n (tested)	S (SDR) n (%)	S (IE) n (%)	R n (%)
PIP	80	-	9 (11.3)	71 (88.7)
TZP	100	1 (1.0)	13 (13.0)	86 (86.0)
FEP	100	-	29 (29.0)	71 (71.0)
AZT	79	-	21 (26.6)	58 (73.4)
AK	100	56 (56.0)	5 (5.0)	39 (39.0)
TOB	75	42 (56.0)	-	33 (44.0)
CIP	100	8 (8.0)	8(8)	84 (84.0)
LEV	91	-	9 (9.9)	82 (90.1)

PIP: Piperacillin, TZP: Piperacillin-tazobactam, FEP: Cefepime, AZT: Aztreonam, AK: Amikacin, TOB: Tobramycin, CIP: Ciprofloxacin, LEV: Levofloxacin

novel antibiotics have been introduced for these superbugs (17). Ceftazidime-avibactam is a novel antibiotic that contains ceftazidime, a broad-spectrum cephalosporin, and avibactam, a non ß-lactam ß-lactamase inhibitor. Ceftazidime-avibactam demonstrates good in vitro activity against members of *Enterobacterales* and *P. aeruginosa* isolates and inactivates Class A ß-lactamases (cephalosporinases, extended-spectrum beta lactamases, etc.), Class C ß-lactamases, and various Class D ß-lactamases (OXA carbapenemases). However, it does not show activity against B-type metallo-enzymes due to the absence of serine residues in that active site (18, 19). In this study, we determined the MIC values of ceftazidime-avibactam and colistin among carbapenem-resistant *P. aeruginosa* isolates.

In general, it is possible that the susceptibility rates of ceftazidime-avibactam are lower for *P. aeruginosa* than for *Enterobacterales*. However, as expected differences could be observed depending on the resistance status of the isolates in the studies and the distribution of resistance genes. In a global surveillance study consisting of 5,716 *P. aeruginosa* isolates, susceptibility to ceftazidime-avibactam was found as 92.4% (MIC_{90}=8mg/L). Ceftazidime-avibactam was effective against colistin-resistant isolates (92.9%) and meropenem-resistant isolates without possessing the acquired beta-lactamases genes (87.6%). In the same study, susceptibility to colistin was found to be 99.6% (20). In another study conducted with one-hundred and two non-meropenem-susceptible *P. aeruginosa* from Turkiye, susceptibility to ceftazidime-avibactam was reported as 83.3% (2). We revealed that ceftazidime-avibactam showed good in vitro activity on carbapenem-resistant *P. aeruginosa* clinical isolates in our institution. Totally, Ten of one hundred carbapenem-resistant *P. aeruginosa* isolates were resistant to ceftazidime-avibactam in the present study.

Colistin is another remarkable agent for systemic MDR Gram-negative bacilli infections such as ventilator-associated pneumonia and bacteremia. It was first discovered in 1947 from the spore-forming bacterium *Paenibacillus polymyxa*. Overuse of colistin may result in the development of high resistance rates, especially in countries where carbapenem-resistant bacteria-related infections are common (21). In general, susceptibility to colistin remains at higher levels among the *P. aeruginosa* isolates. In a report from the International Network for Optimal Resistance Monitoring (INFORM) global surveillance program, colistin (MIC_{90}=2 mg/L, 96.2% susceptibility) was the most potent antibiotic for the tested *P. aeruginosa* isolates (n=11.032). In the same study, ceftazidime-avibactam was found to be the second most effective agent after colistin (MIC_{90}: 8 mg/L; susceptibility: 91.5%) (5). A meta-analysis reviewed *P. aeruginosa* antimicrobial resistance over ten years in Turkiye between 2007 and 2016, and resistance rates to meropenem, imipenem and colistin were 30.1%, 28.0% and 2.2%, respectively. When the authors compared 2007-2011 and 2012-2016 in their study, it was determined that the rates of resistance to piperacillin, piperacillin-tazobactam, imipenem, meropenem, amikacin, and colistin increased significantly in the second five-year period (22). In another study consisting of seventy *P. aeruginosa* isolates, twenty-four of which were carbapenem resistant, from Turkiye, none of the isolates were found resistant to colistin (23). Of 784 carbapenemase-producing *P. aeruginosa* isolates with predominantly MBL carbapenemases obtained from four geographic regions between 2016 and 2018 under the Antimicrobial Testing Leadership and Surveillance (ATLAS) programme, all isolates were reported susceptible to colistin, except for one isolate collected in Latin America. In the same report due to high MBL positivity among the isolates, ceftazidime-avibactam showed lower activity and the resistance rates ranged from 82.5% to 92.3% (24). In a recent study, Karlowsky et al. noticed that 99.7% and 88.2% of 321 *P. aeruginosa* isolates were susceptible to ceftazidime-avibactam and colistin, respectively. Unfortunately, susceptibility of ceftazidime-avibactam decreased to 45.7% among 59 MDR isolates in the same study (25). Antimicrobial susceptibility tests of colistin has some challenges. Antibiotic susceptibility results for colistin may vary depending on the susceptibility testing methods conducted in studies (26). The susceptibility testing method recommended by EUCAST for colistin is broth microdilution. For this reason, it is necessary to conduct studies using this method.

In the present study, we noticed that 100% and 90% of meropenem non-susceptible *P. aeruginosa* isolates were susceptible to colistin and ceftazidime-avibactam, respectively. These findings are extremely satisfactory for our hospital. The main limitation of the present study is that it was conducted in a single center. However, the fact that our study includes non-duplicate carbapenem-resistant *P. aeruginosa* isolates obtained in our center over a five-year period and antibiotic susceptibility testing was assigned by the reference method makes it valuable.

CONCLUSION

Susceptibility patterns of bacteria vary from one region to other, even from one hospital to another. Future studies about antimicrobial activity including MDR isolates should be conducted as multicenter disciplines using long-term surveillance data. In conclusion, our results reveal that ceftazidime-avibactam is a successful alternative therapeutic option against clinical isolates of carbapenem-resistant *P. aeruginosa*. The fact that colistin resistance was not detected in *P. aeruginosa* isolates indicates a satisfactory finding for our institution. Rational

antimicrobial stewardship efforts should be followed to prevent the development of antimicrobial resistance even with last-line therapeutic antibiotics. Antimicrobial susceptibility testing with appropriate method is essential for ceftazidime-avibactam and colistin surveillance.

Ethics Committee Approval: The presented study was approved by the Ethical Committee of the University of Health Sciences (Date: 22.10.2019, No: 19/320).

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