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RESEARCH ARTICLE

Activity of meropenem-vaborbactam against different beta-lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* isolates in Iran

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ABSTRACT

We evaluated the activity of meropenem-vaborbactam against different beta-lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* isolates. In our study antibiotic susceptibility testing, double disk synergy test, modified Hodge test were applied. Detection of ESBL, AmpC, and carbapenemase genes was performed by PCR. Multilocus sequence typing (MLST) analysis was done on OXA-48 producing *K. pneumoniae* strains. Our results showed that among *E. coli* and *K. pneumoniae* isolates, 41.1% and 40% of strains produced ESBL, respectively. Additionally, the prevalence of AmpC producing *K. pneumoniae* and *E. coli* was 4% and 45.5%, respectively. Altogether 64.2% of *K. pneumoniae* strains and one *E. coli* isolate produced carbapenemase. Among OXA-48 producing *K. pneumoniae* strains ST3500 and ST2528 were detected by MLST. Based on the phenotypic results of this study, vaborbactam was an effective inhibitor on the third-generation cephalosporin-resistant isolates ($P < 0.0001$). Meropenem-vaborbactam combination had the highest efficacy on KPC producing strains, and it had limited activity on isolates producing OXA-48 type beta-lactamases, whereas no effect was observed on NDM-1 producing isolates. Our study provided valuable information regarding the vaborbactam inhibitory effect on β-lactamase-producing strains.

KEYWORDS

vaborbactam, KPC, AmpC, ESBL, *K. pneumoniae*, *E. coli*

1. INTRODUCTION

Gram-negative bacilli are the most common causative agents of urinary tract infection [1]. *Escherichia coli* and *Klebsiella pneumoniae* are opportunistic Gram-negative pathogens and cause nosocomial infections including pneumonia, cystitis, soft tissue infections, urinary tract infections, meningitis, surgical wound infections, gastroenteritis, and life-threatening infections such as sepsis and endocarditis [2–4]. The evolution of genetic mechanisms over the years has led to the emergence of resistant strains furthermore, multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacteria are resistant to almost all available antibiotics [5, 6]. These bacteria have become resistant to beta-lactam antibiotics such as carbapenems due to the acquisition of plasmids encoding β-lactamases. For this reason, the treatment of infections caused by such microorganisms is difficult [7]. Carbapenems are often considered the last line of treatment for serious infections caused by MDR organisms, but these agents

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are hydrolyzed by carbapenemases including KPC, OXA-48, and metallo-beta-lactamase (MBL), which are disseminated worldwide [8].

Vaborbactam (formerly RPX 7009) is a cyclic boronic acid and β-lactamase inhibitor that is highly active against the A and C classes of Ambler enzymes [9]. This inhibitor increases carbapenem activity against KPC-producing isolates compared to beta-lactams alone [10]. In 2014, Vaborbactam in combination with meropenem (Vabomere) was approved by the US Food and Drug Administration (FDA) for treating complex and acute urinary tract infections (CUTI), complex intra-abdominal infections (CIAIs), febrile neutropenia, pyelonephritis (AP), and ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP), and catheter associated with blood infections [11].

The aim of this study was to assess the activity of meropenem-vaborbactam (M/V) against the isolates of *E. coli* and *K. pneumoniae* producing different β-lactamases.

2. MATERIALS AND METHODS

2.1. Study isolates

A total of 293 urine samples were collected from the patients admitted to the ICU of Qazvin teaching hospitals during September 2019 to June 2020. Not all patients had taken antibiotics at the time of sampling. Biochemical test API 20E (BioMérieux, France) was used to confirm the identity of isolates.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed based on the Kirby-Bauer method according to the protocol provided by the CLSI 2021. The antibiotic disks (Mast Group, Merseyside, UK) used in our study were as follows: ampicillin (10 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftazidime-clavulanate (10/10 µg), cefotaxime (30 µg), cefotaxime-clavulanate (10/30 µg), cefuroxime (30 µg), cefepime (30 µg), meropenem (10 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), nitrofurantoin (300 µg), and ciprofloxacin (5 µg) [12]. By definition, if a bacterial isolate was non-sensitive to at least one antimicrobial agent in three classes or more, it was called MDR [5]. Boronic acid method was used to identify the AmpC β-lactamase-producing isolates [13]. The combined disk method was also used to identify the extended-spectrum β-lactamase (ESBL) producing strains [14]. *E. coli* ATCC 25922 was used as control strain. The modified Hodge test (MHT) was used to confirm the non-susceptible to meropenem [14]. To investigate the effect of vaborbactam on KPC producing strain, the *K. pneumoniae* ATCC 13883 was used.

2.3. Molecular analysis of β-lactamase genes by PCR

Genomic DNA was extracted by the Gentra Puregene kit (Qiagen, Germany). The frequency of ESBL ($bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV}), AmpC (bla_{FOX}, bla_{MOX}, bla_{DHA}, bla_{CIT}, bla_{ACC}, and bla_{EBC}) and carbapenemase (bla_{VIM}, $bla_{\text{NDM-1}}$, bla_{IMP}, $bla_{\text{OXA-48-like}}$, and bla_{KPC}) related genes were analyzed by PCR. A final mixture volume of 25 µl containing 12.5 µl of 2X master mix (Ampliqon), 2 µl of extracted DNA, 0.6 µl of forward primer, and 0.6 µl of reverse primer was prepared. All primers used are listed in Table 1.

Each PCR reaction cycle included several steps as follows: Pre-denaturating at 94 °C (5 min) for 30 cycles, Denaturating at 94 °C (30 s), annealing (listed in the table) (30 s), extension at temperature 72 °C (30 s), and a final-extension step at 72 °C for 10 min. The PCR product was electrophoresed on 1.2% agarose gel prepared in TBE 0.5X buffer and visualized under ultraviolet (UV) light.

2.4. Multilocus sequence typing (MLST) analysis

Genotyping by MLST analysis was conducted for $bla_{\text{OXA-48-like}}$ and bla_{VIM}-carrying *K. pneumoniae* isolates. Briefly, PCR was carried out for seven housekeeping genes including *gapA*, *infB*, *mdh*, *phoE*, *tonB*, *pgi*, and *rpoB*. Results were analyzed according to the Institute Pasteur *Klebsiella* MLST database at (https://bigsdb.pasteur.fr/*klebsiella*/*klebsiella*.html) [15].

2.5. Evaluating the effect of vaborbactam on ESBL-, AmpC-producing and meropenem-resistant *E. coli* and *K. pneumoniae* isolates

ESBL, AmpC producing and meropenem-resistant *E. coli* and *K. pneumoniae* isolates were cultured on Mueller Hinton agar (Merck, Germany) to evaluate the effect of vaborbactam. The inoculum was prepared at a density of 0.5 McFarland standard (~0.8 at OD_{600}). Briefly, two disks of ceftazidime and two cefotaxime disks were placed at a distance of 3 cm on each plate and one inoculated with 20 µl vaborbactam (8 µl ml^{-1}) and incubated at 37 °C for 18–24 h. The results were evaluated based on the diameter of the inhibition zone. A strain was considered sensitive to vaborbactam when the difference in the diameter of the growth inhibition zone between the disks of ceftazidime and cefotaxime and vaborbactam containing ceftazidime and cefotaxime disks was ≥5 mm [16, 17].

Also, the meropenem-resistant *E. coli* and *K. pneumoniae* isolates were evaluated by disk diffusion method. Briefly, meropenem and meropenem-vaborbactam disks (10/20 µg) were placed on the Muller Hinton agar and all carbapenem non-sensitive strains were analyzed by ETEST method (Liofilchem, Italy) to determine the minimum inhibitory concentration (MIC). Results were evaluated by breakpoints based on CLSI 2021.

2.6. Statistical analysis

Data analysis was performed using SPSS version 22.0. Descriptive results were shown in frequency and mean.

2.7. Ethical approval

The study was approved by the research committee of the Qazvin University of Medical Sciences (Grant No: IR.QU-

Table 1. Primers used in this study

Target gene	Primer name	Primer sequence (5′ → 3′)	Size (bp)	Annealing	References	Positive control
bla_{TEM}	F	TCGCCGCATACACTATTCTC	373	57°C	This study	*K.pneumoniae* ATCC 700603
	R	AACTTTATCCGCCTCCATCC				
bla_{SHV}	F	ATCCACTATCGCCAGCAG	232	53°C	This study	*K.pneumoniae* ATCC 700603
	R	CCTCATTCAGTTCCGTTTCC				
bla_{CTX-M}	F	AGGAAGTGTGCCGCTGTATG	552	57°C	This study	*K.pneumoniae* ATCC 700603
	R	CTGTCGCCCAATGCTTTACC				
bla_{MOX}	F	GCTGCTCAAGGAGCACAGGAT	520	61°C	[17]	*Enterobacter cloacae* 029 M
	R	CACATTGACATAGGTGTGGTGC				
bla_{FOX}	F	AACATGGGGTATCAGGGAGATG	190	61°C	[17]	*Enterobacter cloacae* 029 M
	R	CAAAGCGCGTAACCGGATTGG				
bla_{CIT}	F	TGGCCAGAACTGACAGGCAAA	462	61°C	[17]	*Enterobacter cloacae* 029 M
	R	TTTCTCCTGAACGTGGCTGGC				
bla_{DHA}	F	AACTTTCACAGGTGTGCTGGGT	405	61°C	[17]	*Enterobacter cloacae* 029 M
	R	CCGTACGCATACTGGCTTTGC				
bla_{ACC}	F	AACAGCCTCAGCAGCCGGTTA	346	61°C	[17]	*Enterobacter cloacae* 029 M
	R	TTCGCCGCAATCATCCCTAGC				
bla_{EBC}	F	TCGGTAAAGCCGATGTTGCGG	302	61°C	[17]	*Enterobacter cloacae* 029 M
	R	CTTCCACTGCGGCTGCCAGTT				
bla_{kpc}	F	CGTCTAGTTCTGCTGTCTTG	383	58°C	This study	*K.pneumoniae* ATCC 13883
	R	GCGGCGTTATCACTGTATTG				
$bla_{oxa-48-like}$	F	GGCGTAGTTGTGCTCTGG	487	57°C	This study	*K.pneumoniae* ATCC 13883
	R	TATAGTCACCATTGGCTTCGG				
bla_{NDM-1}	F	ATACCGCCTGGACCGATGAC	395	61°C	This study	*K.pneumoniae* ATCC 13883
	R	GAGATTGCCGAGCGACTTGG				
bla_{VIM}	F	TGTCGCAAGTCCGTTAGC	480	56°C	This study	*K.pneumoniae* ATCC 13883
	R	GCAGCACCAGGATAGAAGAG				
bla_{IMP}	F	TTAGCGGAGTTAGTTATTGGC	335	56°C	This study	*K.pneumoniae* ATCC 13883
	R	TTAGTTACTTGGCTGTGATGG				

MS.REC.1400.077), Qazvin, Iran. As the bacterial isolates were collected as microbiology laboratory in the hospital, ethical approval was not required.

3. RESULTS

3.1. Antimicrobial susceptibility

A total of 165 (56.3%) urinary isolates were collected of which 75 (45.5%) and 90 (54.5%) isolates identified as *K. pneumoniae* and *E. coli*, respectively. MDR phenotype was observed in 60 (80%) and 79 (87.7%) strains of *K. pneumoniae* and *E. coli*, respectively. The highest antibiotic resistance rate was observed for ampicillin (98.7%), ceftazidime (64%), and cefuroxime (62.7%) in *K. pneumoniae* and cefotaxime (90%), ceftazidime (84.5%), and cefoxitin (45.5%) in *E. coli*. The highest susceptibility to antibiotics was revealed for gentamicin (73.3%), meropenem (64%), and cefoxitin (52%) in *K. pneumoniae* and meropenem (98.9%), gentamicin (88.9%), and nitrofurantoin (84.5%) in *E. coli*. The complete results obtained to determine antimicrobial susceptibility are given in Table 2. A total of 28 isolates non-sensitive to meropenem were identified by the disk diffusion method. The isolates were approved by the Hodge test as carbapenemase producers. Of these, 23 isolates were confirmed in *K. pneumoniae* and one in *E. coli*.

3.2. Molecular analysis of antibiotic resistance

Our findings showed that the prevalence rates of ESBL-producing *K. pneumoniae* and *E. coli* were 30 (40%) and 37 (41.1%), respectively. The highest frequency was for bla_{SHV} gene 29 (96.6%) followed by bla_{CTX-M} 23 (76.6%) and bla_{TEM} 20 (66.6%) genes in *K. pneumoniae* while in *E. coli*, the highest frequency was detected for bla_{TEM} gene 36 (97.2%) followed by bla_{CTX-M} 31 (83.7%) and bla_{SHV} 11 (29.7%) genes. Additionally, the prevalence of AmpC related genes in *K. pneumoniae* and *E. coli* was 3 (4%) and 41 (45.5%), respectively. The bla_{DHA} gene was detected in 3 (100%) isolates of *K. pneumoniae*. The genes bla_{CIT} and bla_{DHA} were detected in 41 (100%) and 1 (2.4%) cases of *E. coli* isolates, respectively. The bla_{FOX}, bla_{MOX}, bla_{EBC}, bla_{ACC} genes were not identified in any isolate. Also, bla_{CIT} gene was not detected in *K. pneumoniae*. Five *E. coli* strains carried more than one gene associated with ESBL and AmpC. Three isolates were found to bear bla_{TEM}/bla_{CIT} genes and two isolates showed the presence of bla_{TEM}, bla_{SHV}, bla_{CTX}/bla_{CIT} genes, simultaneously. ESBL and AmpC genes were not identified together in *K. pneumoniae*.

Of 28 non-sensitive carbapenem strains, in 18 (64.2%) isolates were detected carbapenemase genes. The bla_{VIM}, bla_{NDM-1}, and $bla_{OXA-48-like}$ genes were identified in 3 (17.6%), 6 (35.2%), and 15 (88.2%) *K. pneumoniae* isolates, respectively. The bla_{IMP} and bla_{KPC} genes were not detected in *K. pneumoniae*. The $bla_{OXA-48-like}$ gene was detected in

Table 2. Antibiotic resistance patterns of 165 isolates of *K. pneumoniae* and *E. coli*

Antibiotic	No. of isolates of *K. pneumoniae* (%)			No. of isolates of *E. coli* (%)		
	S	I	R	S	I	R
Ceftazidime	25 (33.4%)	2 (2.6%)	48 (64%)	5 (5.5%)	9 (10%)	76 (84.5%)
Cefoxitin	39 (52%)	3 (4%)	33 (44%)	49 (54.5%)	0 (0%)	41 (45.5%)
Cefotaxime	23 (30.7%)	6 (8%)	46 (61.3%)	9 (10%)	0 (0%)	81 (90%)
Meropenem	48 (64%)	7 (9.3%)	20 (26.7%)	89 (98.9%)	0 (0%)	1 (11.1%)
Cefuroxime	10 (13.3%)	18 (24%)	47 (62.7%)	66 (73.3%)	15 (16.7%)	9 (10%)
Cefepime	27 (36%)	6 (8%)	42 (56%)	74 (82.2%)	2 (2.2%)	14 (15.6%)
Ampicillin	0 (0%)	1 (1.3%)	74 (98.7%)	51 (56.7%)	17 (18.9%)	22 (24.4%)
Trimethoprim-sulfamethoxazole	30 (40%)	1 (1.3%)	44 (58.7%)	65 (72.2%)	9 (10%)	16 (17.8%)
Gentamicin	55 (73.3%)	0 (0%)	20 (26.7%)	80 (88.9%)	4 (4.4%)	6 (6.7%)
Nitrofurantoin	30 (40%)	8 (10.7%)	37 (49.3%)	76 (84.5%)	10 (11.1%)	4 (4.4%)
Ciprofloxacin	21 (28%)	10 (13.3%)	44 (58.7%)	75 (83.3%)	4 (4.5%)	11 (12.2%)

one *E. coli* isolate and other carbapenemase genes were not observed in *E. coli*. No carbapenemase genes were identified in the remaining 6 isolates of carbapenem non-sensitive *K. pneumoniae*. The simultaneous presence of several carbapenemase genes was also identified in a number of isolates, shown in Table 3.

3.3. Effect of vaborbactam on β-lactamase producing strains

In *K. pneumoniae* and *E. coli* strains, the rate of resistance to ceftazidime was 48 (64%) and 76 (84.4%), and that of cefotaxime 46 (61.3%) and 81 (90%), respectively. After adding 20 µl vaborbactam to ceftazidime disk, the results were indicative of reduction in inhibition zone in 22 (29.3%) and 41 (45.5%), respectively. Regarding the cefotaxime disk, this reduction was 23 (30.6%) and 41 (45.5%), respectively ($P < 0.0001$).

As shown in Fig. 1, the highest effect of vaborbactam was observed on isolates with bla_{KPC} gene (100%), whereas no effect was observed on isolates with $bla_{\text{NDM-1}}$ gene (0%) and a slight effect on isolates with $bla_{\text{OXA-48-like}}$ gene. Also, the effect of vaborbactam on meropenem resistant isolates in the diffusion disk method showed an increase of 2 mm in inhibition zone of the organisms with $bla_{\text{OXA-48-like}}$ gene carrier isolate.

4. DISCUSSION

Antimicrobial resistance is a global concern that causes high mortality rates. In this regard it is of great importance that the production of β-lactamases by gram-negative bacteria has led to resistance to various β-lactam antibiotics [18]. In the present study, we investigated the activity of meropenem-vaborbactam (M/V) on β-lactamase producing *E. coli* and *K. pneumoniae* isolates *in vitro*. The MDR rates among the *K. pneumoniae* and *E. coli* isolates were 80% and 87.7%, respectively. The rate of MDR isolates of *E. coli* is reported to be around 88% in Iran and the MDR among the isolates of *K. pneumoniae* in India has shown a rate of 75% that were in agreement with our study [19, 20]. In other studies in Iran and Nepal, the number of MDR strains isolated was not in line with our results. In another report from Iran in 2016, the MDR rate for the strains of *E. coli* was 68% whereas in Nepal the figure reported was 52.3% in 2017 [21, 22]. These results suggest that the rate of MDR strains is increasing rapidly due to over-administration of third-generation cephalosporins by physicians. Previous studies have shown that the AmpC and CTX-M-producing strains cause a false-positive MHT test [23, 24], a finding in agreement with the results of the present study. Out of 28 strains non-sensitive to meropenem, 6 strains lacked carbapenemase genes among those 3 strains were detected positive for the presence of AmpC and $bla_{\text{CTX-M}}$ gene. One strain contained the $bla_{\text{CTX-M}}$ gene and two strains carried the bla_{DHA} gene. However, KPC is one of the most common carbapenemases from the carbapenem resistant Enterobacteriaceae (CRE) distributed globally including the United States, Italy, China, Greece, and Brazil [25].

However, in this study, we did not identify the bla_{KPC} gene and we used a positive control strain from the Iranian Internal Standard which contained the bla_{KPC} gene. The most common carbapenemase identified in this study was the $bla_{\text{OXA-48-like}}$ gene. In the recent three studies conducted in Iran on 161 carbapenemase-producing isolates, no bla_{KPC} gene was identified [26–28]. Furthermore, other studies conducted in the Middle East, showed the bla_{KPC} gene is not prevalent in these areas [29, 30]. Vaborbactam, when tested along with various carbapenems, reduced the MICs of these agents against *Klebsiella* spp., *E. coli*, and *E. cloacae* [31]. In a previous study performed on 991 isolates with bla_{KPC} gene, the inclusion of vaborbactam caused MIC reduction from 32 to 0.06 µg ml^{-1} [32]. In two other studies, conducted on 121 and 135 isolates producing KPC, the MIC range was between 0.004 and 64 µg ml^{-1}, and meropenem-vaborbactam showed the best effect at 8 µg ml^{-1} [33, 34]. In our study, the MIC range of meropenem was between 24 and 256 µg ml^{-1} which later reduced to values between 0.25.µg ml^{-1} to 256 µg ml^{-1} when the effect of combined meropenem-vaborbactam was evaluated. Of the 28 isolates non-sensitive to meropenem, 3.4% showed a MIC <2 µg ml^{-1} (KPC producing isolates) after the addition of vaborbactam.

Table 3. Frequency of β-lactamase genes and MIC values of MER and M/V in isolates non-sensitive to meropenem

Bacterial	H.T	Carbapenemase genes	MLST	ESBL/AmpC	disk diffusion Mer/ M/V	MIC (ETEST) MER	MIC (ETEST) M/V
K. P 1	+	$bla_{\text{OXA-48-like}}$, bla_{VIM}	ST-3500	bla_{TEM} 'bla_{SHV} '$bla_{\text{CTX-M}}$	R/R	32 μg ml^{-1}	16 μg ml^{-1}
K. P 2	+	$bla_{\text{OXA-48-like}}$	ST-2528		R/R	24 μg ml^{-1}	16 μg ml^{-1}
K. P 3	+	$bla_{\text{OXA-48-like}}$, bla_{VIM}		-	R/R	24 μg ml^{-1}	16 μg ml^{-1}
K. P 4	+	$bla_{\text{OXA-48-like}}$		bla_{TEM} 'bla_{SHV} '$bla_{\text{CTX-M}}$	R/R	32 μg ml^{-1}	24 μg ml^{-1}
K. P 5	+	$bla_{\text{NDM-1}}$		-	R/R	256< μg ml^{-1}	256< μg ml^{-1}
K. P 6	+	$bla_{\text{OXA-48-like}}$, $bla_{\text{NDM-1}}$		-	R/R	96 μg ml^{-1}	64 μg ml^{-1}
K. P 7	+	$bla_{\text{OXA-48-like}}$	ST-2528	bla_{TEM} 'bla_{SHV} '$bla_{\text{CTX-M}}$	R/R	48 μg ml^{-1}	32 μg ml^{-1}
K. P 8	+	$bla_{\text{OXA-48-like}}$, $bla_{\text{NDM-1}}$		-	R/R	96 μg ml^{-1}	64 μg ml^{-1}
K. P 9	+	$bla_{\text{OXA-48-like}}$, $bla_{\text{NDM-1}}$		-	R/R	96 μg ml^{-1}	64 μg ml^{-1}
K. P 10	+	$bla_{\text{OXA-48-like}}$		-	R/R	32 μg ml^{-1}	24 μg ml^{-1}
K. P 11	+	$bla_{\text{OXA-48-like}}$		-	R/R	24 μg ml^{-1}	16 μg ml^{-1}
K. P 12	+	$bla_{\text{OXA-48-like}}$, bla_{VIM}		-	R/R	32 μg ml^{-1}	16 μg ml^{-1}
K. P 13	+	$bla_{\text{OXA-48-like}}$		-	R/R	32 μg ml^{-1}	24 μg ml^{-1}
K. P 14	+	$bla_{\text{OXA-48-like}}$, $bla_{\text{NDM-1}}$		-	R/R	96 μg ml^{-1}	64 μg ml^{-1}
K. P 15	+	$bla_{\text{OXA-48-like}}$		-	R/R	32 μg ml^{-1}	24 μg ml^{-1}
K. P 16	+	$bla_{\text{OXA-48-like}}$, $bla_{\text{NDM-1}}$		-	R/R	96 μg ml^{-1}	64 μg ml^{-1}
K. P 17	+	bla_{KPC}^{*}		bla_{TEM} 'bla_{SHV} '$bla_{\text{CTX-M}}$	R/S	256< μg ml^{-1}	0.25 μg ml^{-1}
K. P 18	+	-		-	R/R	32 μg ml^{-1}	32 μg ml^{-1}
K. P 19	+	-		bla_{TEM} 'bla_{SHV} '$bla_{\text{CTX-M}}$	R/R	24 μg ml^{-1}	24 μg ml^{-1}
K. P 20	+	-		bla_{DHA}	R/R	24 μg ml^{-1}	16 μg ml^{-1}
K. P 21	+	-		-	R/R	24 μg m^{-1}	16 μg ml^{-1}
K. P 22	+	-		_	R/R	24 μg ml^{-1}	24 μg ml^{-1}
K. P 23	+	-		bla_{DHA}	R/R	24 μg ml^{-1}	16 μg ml^{-1}
E. coli	+	$bla_{\text{OXA-48-like}}$		-	R/R	32 μg ml^{-1}	24 μg ml^{-1}

* Positive control.
Mer: Meropenem.
M/V: Meropenem/Vaborbactam

Also, one of the findings of the present study, which is reported for the first time, is the effect of vaborbactam on isolates containing two concurrent carbapenemase genes. Our results showed that although vaborbactam had no effect on isolates containing $bla_{\text{NDM-1}}$ gene, it had a slight effect on isolates containing $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ genes, causing the MIC to reduce from 256 <μg/ml to 64 μg ml^{-1}.

Some studies have shown that *K. pneumoniae* sequence types (STs) are associated with the presence of the $bla_{\text{NDM-1}}$ gene [35]. In the present study also showed that the $bla_{\text{OXA-48}}$ and bla_{VIM} genes are present in the ST-3500 and ST-2528.

A previous study showed that vaborbactam acts as a potent inhibitor of classes A and C β-lactamase, a finding in agreement with the results of this study [36]. In a study by Tsivkovski et al., vaborbactam was a weak inhibitor of class D β-lactamases with no inhibition over the metallo β-lactamases (VIM and NDM-1) [37]. In this study, the highest inhibitory power of vaborbactam was on the isolates producing KPC, ESBL, and AmpC gene and with a minimal effect on class D serine β-lactamase but with no effect on class B metallo-β-lactamase. Therefore, the application of vaborbactam along with meropenem showed an inhibitory effect on class A and C β-lactamase-producing isolates. Similarly, Hackel et al. assessed the activity of meropenem-vaborbactam on isolates producing ESBL and AmpC β-lactamases, and concluded that this compound has a strong effect on the activity of these enzymes [32]. In this regard, our results showed that vaborbactam had the greatest effect on SHV-producing strains in *K. pneumoniae* and TEM-producing strains in *E. coli*.

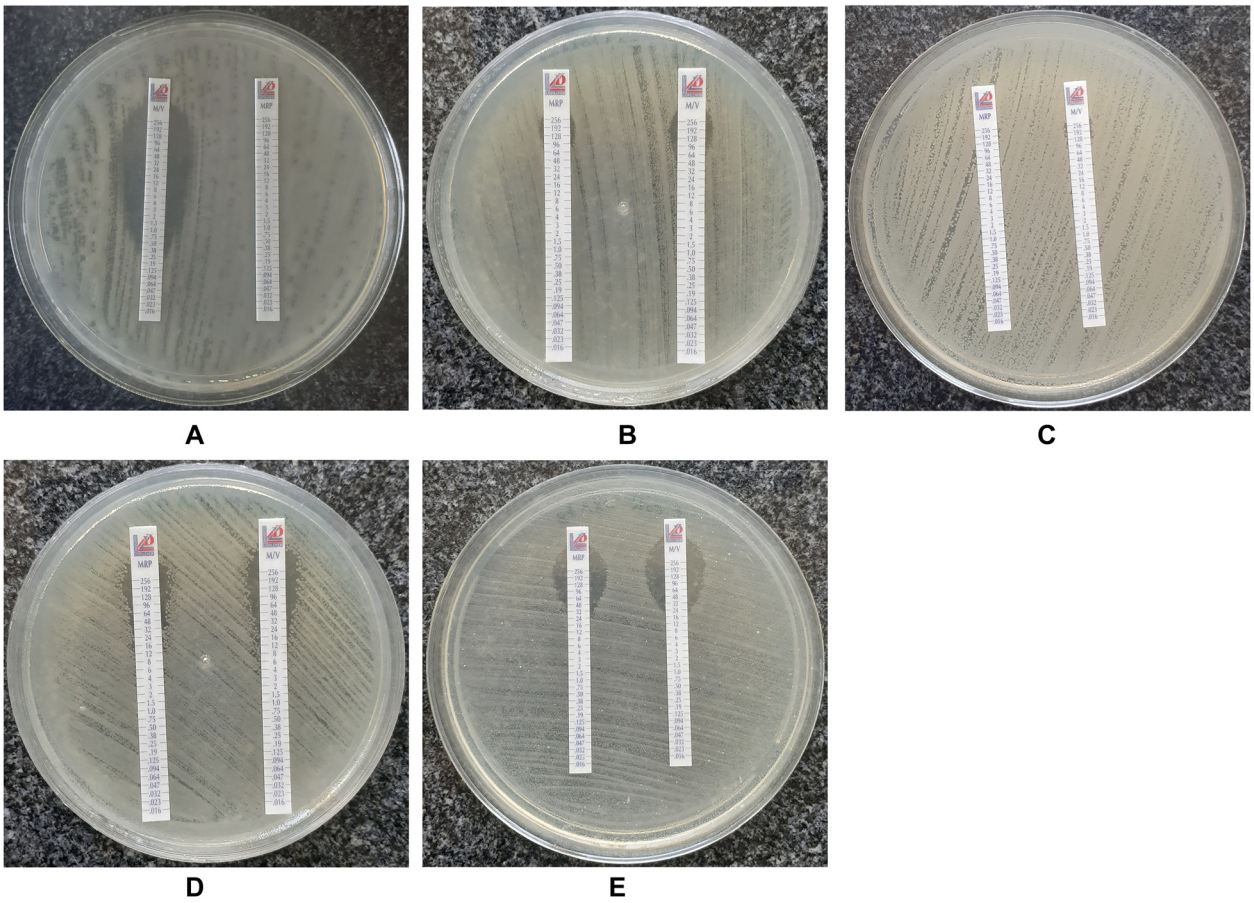

Fig. 1. A) The image shows the result of a Meropenem (M) and Meropenem/Vaborbactam (M/V) ETEST, where the KPC producing-isolate is sensitive to M/V (MIC = 0.25 μg ml^{-1}). B) The image shows the result of a M and M/V ETEST, where the NDM-1 producing isolate is resistant to M/V (MIC 256<μg ml^{-1}). C) The image shows the result of a M and M/V ETEST, where the OXA-48 producing isolate is resistant to M/V (MIC = 32 μg ml^{-1}). D) The image shows the result of a M and M/V ETEST, where the OXA-48 and VIM producing isolate is resistant to M/V (MIC = 16 μg ml^{-1}). E) The image shows the result of a M and M/V ETEST, where the OXA-48 and NDM-1 producing isolate is resistant to M/V (MIC = 64 μg ml^{-1})

5. CONCLUSION

As the bacteria isolated from the ICUs are MDR or even XDR strains, the treatment options are often limited. Therefore, new approaches are needed to enter the treatment protocol of these patients. Our results showed that vaborbactam had significant effect on *K. pneumoniae* and *E. coli* strains producing class A and C β-lactamases and with minimal effect on β-lactamases of class D. These results can be important in controlling the infection caused by these gram-negative bacilli in hospitals.

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