Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 2 Vol 8 No 1 2023

Article

Effect of Berberine as efflux pump inhibitor in multidrug-resistant *Klebsiella pneumoniae* isolated from urinary tract infections

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.02.73

Abstract

The urinary tract infections with *K. pneumoniae* have increased over many years. The emergence of antibiotic resistance among these bacteria presents a challenging problem for the clinician regarding the management and treatment of infections. The multidrug resistance in *K. pneumoniae* is due to several mechanisms, one of which is the role of efflux pumps. The current study investigated the role of Efflux Pump Inhibitors Phenylalanine-Arginine β-Naphthylamide (PAβN) and Berberine as antibacterial agents with multidrug-resistant *K. pneumonia* isolates from urinary tract infections. The collection of study samples took place between December 2021 and completed at the end of April 2022; it included 260 urine samples collected from outpatients and inpatients suffering from urinary tract infections during this period, from both genders with ages ranging from 15 to 72 years in five hospitals in Baghdad. The results of selective media, biochemical tests, and the VITEK2 system identified 76 isolates (65.5%) as *K. pneumoniae* from all collected bacterial cultures. The results of the antimicrobial susceptibility test using the disc diffusion method for the isolates under study showed that *K. pneumoniae* clinical isolates were moderately resistant to most antibiotics tested. Most *K. pneumoniae* isolates were highly resistant to Amoxicillin (96.1%) and Trimethoprim (80.3%). Also, there was apparent resistance to Gentamicin and Amikacin, while the lowest percentage of resistance was for Meropenem (55.1%) and Ciprofloxacin (53.9%). The susceptibility of the strains to Ciprofloxacin was highly increased in the presence of the efflux pump inhibitor (PAβN). The PAβN reduced the minimum inhibitory concentrations (MICs) by 4- to 64-fold. The results of minimum inhibitory concentrations (MICs) of Berberine against ten *K. pneumoniae* isolates with multidrug resistance revealed that the range of MICs of Berberine was (3.9-500 µg/ml) and it was obvious that there is a significant effect of Berberine on the growth of *K. pneumoniae* at deficient concentrations. This study concluded that using Berberine as an efflux pump inhibitor and antimicrobial agent may become a new generation of urgently needed antimicrobials that can overcome bacterial resistance mechanisms.

Keywords: UTI infections, MDR, Berberine, *Klebsiella pneumoniae*

Introduction

The incidence of urinary tract infections (UTIs) caused by *Klebsiella pneumoniae* has increased and has become a burden for many public health systems,

especially in hospital settings [1]. Klebsiella pneumoniae could acquire different mechanisms that lead to antibiotic resistance to many antibiotics. 2 The Multidrug resistance (MDR) is highly disseminated among Klebsiella pneumoniae isolates, causing severe problems at the clinical sites due to the decreased therapeutic options available to treat such resistant bacteria [3]. Klebsiella pneumoniae is a Gram-negative, rod-shaped, capsulated bacterium that belongs to the family Enterobacteriaceae [4]. This species has an important opportunistic pathogen causing nosocomial infections, especially in urinary, respiratory tracts, and blood [5]. Multidrug efflux pumps play a significant role as a mechanism of antimicrobial resistance in Gram-negative pathogens. Efflux systems significantly confer intrinsic and acquired resistance to the bacteria [6]. Among the efflux pumps, the resistance-nodulation-division (RND) family, including OqxAB and AcrAB, are essential in creating antibiotic resistance in Gram-negative bacteria, especially K. pneumoniae [7]. inhibiting these efflux pumps might seem like an effective strategy at times when the conventional antibiotics remain no longer effective. Establishing natural substrates and efflux pump inhibitors (EPIs) that enable the effective accumulation of drugs inside the bacterial cell enhances the antibacterial activity. Some of the plant-derived EPIs used are Berberine and Reserpine [8]. Considerably used synthetic efflux inhibitors to detect the efflux activity in Klebsiella pneumoniae are carbonyl cyanide-chlorophenylhydrazone (CCCP) and phenylalanine-arginine β-naphthylamide (PAβN) [9].

The present study aimed to investigate the efficacy of Berberine against clinical K. pneumoniae isolates and evaluate the role of this natural efflux pump inhibitor as an alternative therapeutic agent for multidrug-resistant strains in patients with UTIs.

Materials And Methods

Isolation and identification of K. pneumoniae

This study was performed at Hospitals in Baghdad, Iraq, between December 2021 and April 2022. Out of 260 urine samples, 76 isolates of K. pneumoniae were collected from patients with burns infections. CHROM agar Orientation, Blood agar and MacConkey agar were used to isolate K. pneumoniae. According to the manufacturer's recommendations, these isolates were identified using traditional bacteriological methods and biochemical testing with the VITEK 2 system (bioMerieux, France).

Antibiotic Susceptibility Test

An antimicrobial susceptibility test was conducted by using the disc diffusion method. Briefly, K. pneumoniae overnight growth was prepared on MacConkey agar and then resuspended in Mueller-Hinton broth (Himedia). The turbidity of the suspension is adjusted to an equivalent 0.5 McFarland, and this suspension was used to inoculate on Mueller-Hinton agar (Himedia) plates. The antibiotics discs used in this study as the following: Amikacin (AK), Gentamicin (CN), Imipenem (IPM), Meropenem (MEM), Ceftriaxone (CRO), Cefoxitin (CX), Ciprofloxacin (CIP), Levofloxacin (LEV), Amoxicillin/clavulanic acid (AMC), Trimethoprim (SXT), Cefepime(FEP) and Piperacillin (PIT), Bioanalyse /Turkey) were placed on the medium. The agar plates were incubated at 35 °C for 24 h. Then, the inhibition zone was measured and interpreted by the percent of susceptible, intermediate, or resistant isolates as defined by CLSI breakpoint interpretative Criteria (CLSI, 2020).

Minimum inhibitory concentrations (MICs) of Klebsiella pneumoniae isolates

The minimum inhibitory concentrations (MICs) of Klebsiella pneumoniae against eight of the tested antibiotics (Amikacin (AK), Gentamicin (CN), Imipenem (IPM), Meropenem (MEM), Ciprofloxacin (CIP), Levofloxacin (LEV), Cefepime (FEP) and Ceftriaxone (CRO)) were determined via the broth microdilution method in accordance with the guidelines of the CLSI (2020). Escherichia coli ATCC 25922 was used as a quality control strain. According to the manufacturer's recommendations, antibiotic powders were dissolved in appropriate solvent or sterile deionized water. The serial dilutions tested for the antibiotics were 256, 128, 64, 32, 16, 8, 4 and 2 µg/mL. Each well of a 96-well microtiter plate contained a total volume of 100 µL of the antibiotic dilution and Müller-Hinton broth. Subsequently, the 0.5 McFarland suspensions were diluted 1 20 to yield 5×10^6 CFU/mL. When 0.01 mL of this suspension was inoculated into the broth, the final test concentration of the bacteria was approximately 5×10^5 CFU/mL. The correct density of the turbidity standard was verified by measuring absorbance using a spectrophotometer. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standards. The samples were incubated at 37°C for 24 hours. The lowest concentration of the antibiotics that did not have visible bacterial growth was defined as the MIC.

Treatment of the Efflux Pump Inhibitors

Changing susceptibility to antibiotics in the presence of 100 µg/mL of the PABN (BACHEM/USA) was tested as described under "Susceptibility Testing" above. Specifically, susceptibility to antibiotics was tested in parallel with the presence or absence of the PABN. Following the addition of antibiotics and the bacterial cell inoculum, 2 µL of the 5 mg/mL stock of PABN was added to the microplate wells. (Total volume, 100 µL). The rest of the procedures were carried out as described above. To check the acridity of the test and evaluate the effect of the PABN on bacterial growth, all the bacteria were cultured in the Mueller Hinton broth containing the PABN (100 µL/mL). 1:2 serial dilutions of Berberine HCL in Mueller Hinton Broth (MHB) were placed in a 96-well round-bottom plate at concentrations ranging from 3.9 to 500 µg/ml. The bacterial inoculum was prepared from a subculture of K. pneumoniae in LBB and incubated for 18–24 hours at 35 ± 2°C before the test. The bacteria suspension was diluted to $1x10^8$ colony forming units (CFU)/mL to obtain turbidity equivalent to 0.5 on the McFarland scale, confirmed by spectrophotometry upon reaching an absorbance between 0.08–0.1 at a wavelength of 625 nm. A 1:200 dilution in MHB was performed to obtain a final concentration of $5x10^5$ CFU/mL. The diluted bacterial suspension was added to the 96-well plate containing the serially diluted peptides. The final volume of 200 µL per well consisted of 100 µl of the compound and 100 µL of diluted bacteria suspension. Negative and positive growth controls were performed by adding only MHB or K. pneumoniae with MHB to the wells, respectively. After incubation for 24 h at 37 °C, resazurin (0.015 %) was added to all wells (20 µl per well) and further incubated for 2–4 h to observe color change. On completion of the incubation, columns with no color change (blue resazurin color remained unchanged) were scored as above the MIC value. MIC was determined at the end of the incubation time as the lowest compound concentration at which no bacterial growth was observed.

Results

Isolation and characterization of K. pneumoniae

This study was conducted among patients suspected of UTI visiting 5 Baghdad hospitals. Two hundred sixty non-repetitive MSU samples were collected from patients for urine culture. All these urine specimens were cultured on CHROMgarOrientation medium, MacConkey agar and Blood agar plates for 24 hours, and at 37 °C, Only 116 (44.6%) samples showed significant growth. Seventy-six positive cultures were identified as K. pneumoniae (65.5. %). Most of the patients were of the females 50/76(65.7%), while the percentage of the males was 26/76 (34.2 %). K. pneumoniae bacteria growing on MacConkey agar were bright pink colonies with a mucoid structure, which is a characteristic feature of these bacteria (Figure 1).

Figure 1. *Klebsiella* pneumoniae on MacConkey agar plate.

All suspected Klebsiella colonies were detected by culturing on blood agar medium, showing large, shiny, mucoid, whitish-grey and round colonies with no hemolysis. On CHROMagar Orientation, isolates of Klebsiella appeared as metallic blue colonies at 37°C for 24 hours, as shown in Figure (2).

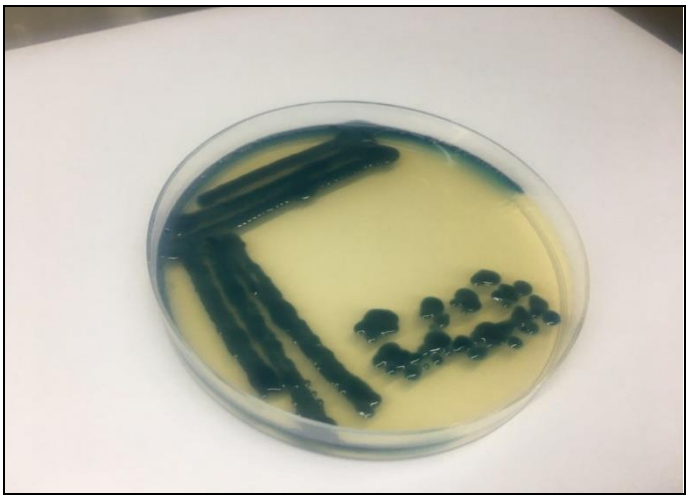

Figure 2. Colonies of *Klebsiella* on CHROMagar Orientation medium.

CHROMagar Orientation medium was used for specific isolation of urinary tract pathogens; this medium also has selectivity for other Urinary tract pathogens with a specific color for each bacterial genus, where the colonies of Escherichia coli appeared as pink-red colonies. Chromogenic agars are reliable for detecting aerobic Gram-negative bacteria by quickly recognizing different colonies on these media. CHROM agar Orientation medium is the preferred medium because of the high accuracy and the rapid identification with meager false favorable rates [11]. The use of CHROM agar Orientation medium reduces the need for further reagents and extra confirmatory tests, suggesting that CO medium is a cost-effective replacement for conventional urine culture methods and its significantly reduced workload in the microbiology laboratory compared to that for Blood agar and MacConkey agar and should be considered as an alternative to conventional culture methods for detecting and reporting uropathogens [12,13].

Klebsiella pneumoniae showed non-hemolytic grey-white mucoid colonies when plated on blood agar. All isolates were non-motile and presented negative results for oxidase, indole, motility, and methyl red tests. In contrast, the isolates showed positive results for urease, citrate utilization, catalase and Voges-Proskauer tests. Glucose was fermented with acid and gas production; H_2S was not produced. (Table 1)

ID	Biochemical test		The positive result of *K. pneumonia*
1	Gram stain		Negative
2	Motility		Negative
3	Indole		(-) ve
4	Simmon citrate test		(+) ve
5	Urease test		(+) ve
6	KIA	H_2S	(-) ve
7		CO_2	(+) ve
8		Acid	A/A
9	Methyl Red test		(-) ve
10	Voges Proskauer test		(+) ve
11	Catalase Test		(+) ve
12	Oxidase		(-) ve
13	String		Positive (≥ 5mm)

Table 1. Biochemical test results of Klebsiella pneumoniae isolates.

The VITEK 2 system was used for the identification of Klebsiella spp. Bacteria with accuracy and reliability of bacterial identification. This device diagnoses bacteria with an accuracy of 99%. After identification, the number of K. pneumonia isolates was 76. The tests used in this system confirmed the results obtained from morphological and biochemical, so all isolates (76) previously identified as Klebsiella spp. are proved to be Klebsiella pneumoniae.

Antibiotic Susceptibility test of Klebsiella pneumoniae isolates

Antimicrobial susceptibility was performed on all 76 K. pneumoniae isolates to 12 antibiotics: Amikacin, Gentamicin, Imipenem, Meropenem, Trimethoprim, Ceftriaxone, Ciprofloxacin, Levofloxacin, Amoxicillin-clavulanic acid, Piperacillin, Cefepime, Cefoxitin as shown in Table (2).

Antibiotic (ug/disc)	**Lev-5**	**CX-30**	**IPM-10**	**FEP-30**	**SXT-25**	**PIT-30**	**AK-30**	**Cip-10**	**MEM-10**	**CN-10**	**AMC-10**	**CRO--30**
S	**45 (59.2%)**	**33 (42.4%)**	**34 (44.7%)**	**33 (42.4%)**	**13 (17.1%)**	**38 (50.0%)**	**28 (36.8%)**	**41 (53.9%)**	**42 (55.2%)**	**22 (28.9%)**	**1 (1.3%)**	**6 (7.9%)**
R	**16 (21.1%)**	**24 (31.6%)**	**33 (42.4%)**	**40 (52.6%)**	**61 (80.3%)**	**30 (39.5%)**	**44 (58.9%)**	**29 (38.2%)**	**27 (35.5%)**	**45 (59.2%)**	**73 (96.1%)**	**66 (8.7%)**
I	**15 (19.7%)**	**19 (25.0%)**	**9 (11.8%)**	**3 (3.9%)**	**2 (2.6%)**	**8 (10.5%)**	**4 (5.3%)**	**6 (7.9%)**	**7 (9.2%)**	**9 (11.8%)**	**2 (2.6%)**	**4 (5.3%)**
Chi-Square- χ^2	**23.16 ****	**4.02 NS**	**15.98 ****	**30.81 ****	**78.50 ****	**19.25 ****	**32.33**	**25.23 ****	**24.59 ****	**26.51 ****	**135.9 ****	**98.99 ****
P-value	**0.0001**	**0.133**	**0.0003**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
**** (P≤0.01).**												

R: Resistant, S: sensitive, I: intermediate.

Table 2. Antibiotic susceptibility results for 76 K. pneumoniae isolates against 12 antibiotics. (Amikacin (AK), Gentamicin (CN), Imipenem (IPM), Meropenem (MEM), Trimethoprim (SXT), Ceftriaxone (CRO), Ciprofloxacin (CIP), Levofloxacin (LEV), Amoxicillin-clavulanic acid (AMC), Piperacillin (PIT), Cefepime (FEP) and Cefoxitin (CX)).

The results of this study showed that the highest percentage of sensitivity of antibiotics against K. pneumoniae was for Levofloxacin (59.2%), Meropenem (55.2%) and Ciprofloxacin (53.9%), while the lowest percentage was for Amoxicillin (1.3%) Ceftriaxone (7.9%) and Trimethoprim (17.1%), while the highest percentage of antibiotic resistance against K.pneumoniae was for Amoxicillin (96.1%), Trimethoprim (80.3%), Gentamicin (59.2%) and Amikacin (58.9%). The antibiogram results showed significant resistance to most antibiotics used in this study. Among 76 isolates of K. pneumoniae, 15(19.7 %) were resistant to more than 3 classes of selected antibiotics, such as Multidrug-Resistant (MDR) K. pneumoniae isolates.

A local study to determine the resistance of K. pneumoniae to 10 antibiotics showed that the highest resistance was against Ampicillin, Cefotaxime, and Piperacillin by 100% for each. In contrast, the rest of the antibiotics had a resistance percentage of 80% for Trimethoprim, 54% for Gentamicin and 65% for Azithromycin. However, the lowest resistance was found against Imipenem, Chloramphenicol and Ofloxacin by 4%, 12. % and 35%, respectively (Ahmed et al., 2020). Our results were consistent with Vasaikar et al. (2017) observation, who found that the resistance of K. pneumoniae isolates isolated from several hospitals in South Africa was 29.7% for Ciprofloxacin, 51% for Gentamicin and 70.8 for Trimethoprim. Another study showed the Antibiogram of K. pneumoniae isolates, where the rate of resistance to Ciprofloxacin, norfloxacin, Gentamicin, kanamycin, cefotaxime, Trimethoprim, chloramphenicol, and colistin was 19.09%, 21.81%, 10.0%, 9.09%, 44.54%, 25.45%, 11.81%, and 61.81%, respectively. After analyzing the antibiotic resistance pattern of the K. pneumoniae samples, 22 multidrug-resistant strains resistant to more than three antibiotics and fluoroquinolones were identified and isolated (Razavi et al., 2020).

Minimum Inhibitory Concentrations (MICs) of K.pneumoniae clinical isolates in the presence and absence of Efflux Pump Inhibitor (Phe-Arg-β- naphthylamide)

The minimum inhibitory concentrations (MICs) for 8 antibiotics were determined using the microdilution method. The results of MIC confirmed the previous results of the antibiotics diffusion test, where the current study showed the high-level resistance of most isolates to the different antibiotics used, except Ciprofloxacin and Meropenem (Table 3). Some high-level resistant isolates recorded the highest MIC values for the antibiotics Amikacin, Gentamicin and Ceftriaxone (265 µg/ml). In contrast, some isolates showed low values for Levofloxacin, Ciprofloxacin and Meropenem (2 and 4 µg/ml).

Antibiotic*	MIC** range (µg/mL)
AK	16 - 256
CN	4 – 256
IPM	2 – 128
MEM	4- 32
LEV	2 – 64
CRO	4 – 256
FEP	16 – 128
CIP	4 – 64

Table 3. The ranges of Minimum inhibitory concentrations (MICs) of different antibiotics for 10 *K. pneumoniae* isolates from urinary tract infections against eight antibiotics. *Amikacin (AK), Gentamicin (CN), Imipenem (IPM), Meropenem (MEM), Ciprofloxacin (CIP), Levofloxacin (LEV), Cefepime (FEB) and Ceftriaxone (CRO), **MIC for each antibiotic was determined twice by Microdilution method In Mueller-Hinton broth. CFU/ml was kept at 1.5×10^8.

[17] found that the MIC values of ceftriaxone were >128 µg/mL in 29 resistant K. pneumoniae strains, and values of imipenem were 128 µg/mL in 28 resistant K.

pneumoniae strains. Carbapenem antibiotics play an essential role in treating severe infections of drug-resistant Enterobacteriaceae, and the increase of drug resistance of K. pneumoniae and the emergence and spread of drug-resistant strains pose a serious threat to public health [18]. Meropenem belongs to the carbapenem class of antibiotics. It is a widely used antibiotic for treating K. pneumoniae infections, with broad-spectrum in vitro resistance to both Gram-positive and Gram-negative pathogens [19]. In a previous study, the ciprofloxacin treatments showed a significant reduction in the number of K. pneumoniae cells compared to meropenem, and the MIC values of Ciprofloxacin and meropenem were 0.03 and 0.06 µg/ml, respectively [28] So the results indicate that meropenem was less effective against K. pneumoniae than Ciprofloxacin. The lack of antibacterial activity of meropenem might be due to its short elimination half-life [20].

In the present study, we evaluate the MICs of 8 antibiotics in the presence of 20 µg/ml of PAβN as a synthetic efflux pump inhibitor and then compared the MICs without PAβN; as the above findings, the effect of Phe-arg-beta-naphthylamide (PAβN) on MICs of K. pneumoniae was illustrated in the **Table (4).**

It was found that the addition of the efflux pump inhibitor (PAβN) at a final concentration of 20 µg/ml significantly reduced the effect of PAβN on MICs of fluoroquinolone (Ciprofloxacin and Levofloxacin) antibiotics, and it was obvious that the reduction of MICs for these antibiotics from 4 to 64 fold. Also, there was a significant reduction of MICs for Amikacin from 4 to 32 fold and Gentamicin from 8 to 32 fold. It was noted that after exposure to the efflux pump inhibitor, a 1 to 4-fold reduction in the MICs of carbapenems was observed. The minimal effect of the inhibitor was correlated with the antibiotics Imipenem, Cefepime, Ceftriaxone and Meropenem.

Iso-late		LE V.	CIP	IPM	ME M.	CN	AK	CRO	FEP
K 1	Alon	2	32	64	32	128	256	64	128
	+EPI	0.	0. 5	32	16	32	16	16	32
K 3	Alon	4	64	64	32	128	64	64	64
	+EPI	0.	1	32	32	16	8	4	32
K 4	Alon	64	64	32	8	256	128	256	32
	+EPI	4	4	8	4	32	16	16	8
K 7	Alon	8	16	64	32	128	64	128	64
	+EPI	0.	1	32	16	64	4	16	64
K 8	Alon	32	64	128	16	64	16	32	16
	+EPI	4	4	128	8	16	0.5	16	1
K1 1	Alon	16	16	64	32	128	64	128	32
	+EPI	0.	0.5	64	32	32	16	32	4
K12	Alon	16	4	4	8	16	64	4	16
	+EPI	0.	0.25	2	4	8	2	1	4
K13	Alon	4	8	32	16	32	32	32	64
	+EPI	0.	1	32	4	0.5	4	16	8
K14	Alon	32	4	2	4	4	16	64	16
	+EPI	8	0.25	2	2	1	4	16	16
K15	Alon	8	8	16	16	32	32	4	16
	+EPI	0.	1	16	8	4	8	0.5	8

Fold of re-duction	**4-64**	**8-64**	**1-4**	**1-4**	**2-64**	**4-32**	**2-16**	**1-16**

MIC, Minimum inhibitory concentration; EPI=Efflux Pump Inhibitor (Phe-Arg-β- naphthylamide). Imipenem(IPM);Gentamicin(CN);Cefepime(FEB);Ceftriaxone(CRO);Amikacin(AK);Meropenem(MEM);Ciprofloxacin(CIP);Levofloxacin(LEV).

Table 4. Antimicrobial susceptibility (MICs) in the presence and absence of Efflux Pump Inhibitor (Phe-Arg-β- naphthylamide) of K. pneumoniae clinical isolates.

It was demonstrated that adding the PAβN reduced the MICs of the 2 classes of antibiotics, fluoroquinolones and aminoglycosides.

The intracellular concentrations of antibiotics in bacteria are directly reduced by active efflux pump systems, leading to decreased antibiotic susceptibility. The inhibition of efflux pumps can restore antibiotic susceptibility in resistant bacteria, suggesting that efflux pump inhibitors can be a possible control method against antibiotic-resistant bacteria. The activation of efflux pumps is associated with the fast-acting antibiotic mechanism at the early stage of antibiotic resistance development. The substrate-dependent efflux pump systems may contribute to cross-resistance to different classes of antibiotics (Kim et al., 2016).

The efflux pump mechanism, AcrAB/TolC, significantly contributes to antibiotic resistance in *K. pneumoniae* strains; thirty-nine percent of the strains exhibited a PAβN-modulated resistance for quinolones, chloramphenicol, and tetracycline. In these strains, a significant increase in chloramphenicol accumulation was gained in the presence of the efflux pump inhibitor PAβN (Hasdemir et al., 2004).

Minimum Inhibitory Concentrations (MICs) of K.pneumoniae clinical isolates in the presence of Berberine

The minimum Inhibitory Concentration (MIC) of Berberine was determined by the Muller Hinton broth micro-dilution method in 96-well microplates. The results of the minimum inhibitory concentrations of antimicrobial Berberine HCL against ten isolates of Klebsiella pneumoniae revealed that there was a difference in MICs between the isolates, where some isolates, such as K1, K26 and K129, were affected by concentrations only (500ug/ml). In contrast, other isolates, such as K2 and K3, were inhibited from the lower concentrations (15.6- 500 ug/ml) (Table 5 and Figure 3).

The results demonstrated that all ten tested isolates were inhibited by the concentration of 500 ug/ml of Berberine, and 7 isolates were inhibited by the concentration of 250 ug/ml. The low concentration of 15.6 ug/ml showed an inhibitor effect on 2 isolates (K2 and K3).

The Isolate code	Minimum Inhibitory Concentration (MIC) (µg/ml)							
	500	250	125	62. 5	31.25	15.6	7.81	3.9
K1	-	+	+	+	+	+	+	+
K2	-	-	-	-	-	-	+	+
K3	-	-	-	-	-	-	+	+
K6	-	-	+	+	+	+	+	+
K7	-	-	+	+	+	+	+	+
K23	-	-	+	+	+	+	+	+
K24	-	-	+	+	+	+	+	+
K26	-	+	+	+	+	+	+	+
K27	-	-	-	+	+	+	+	+
K129	-	+	+	+	+	+	+	+

(+ = Growth; - = No growth (inhibition))

Table 5. The minimum inhibitory concentrations of Berberine against Klebsiella pneumoniae isolates at concentrations (3.9-500 µg/ml).

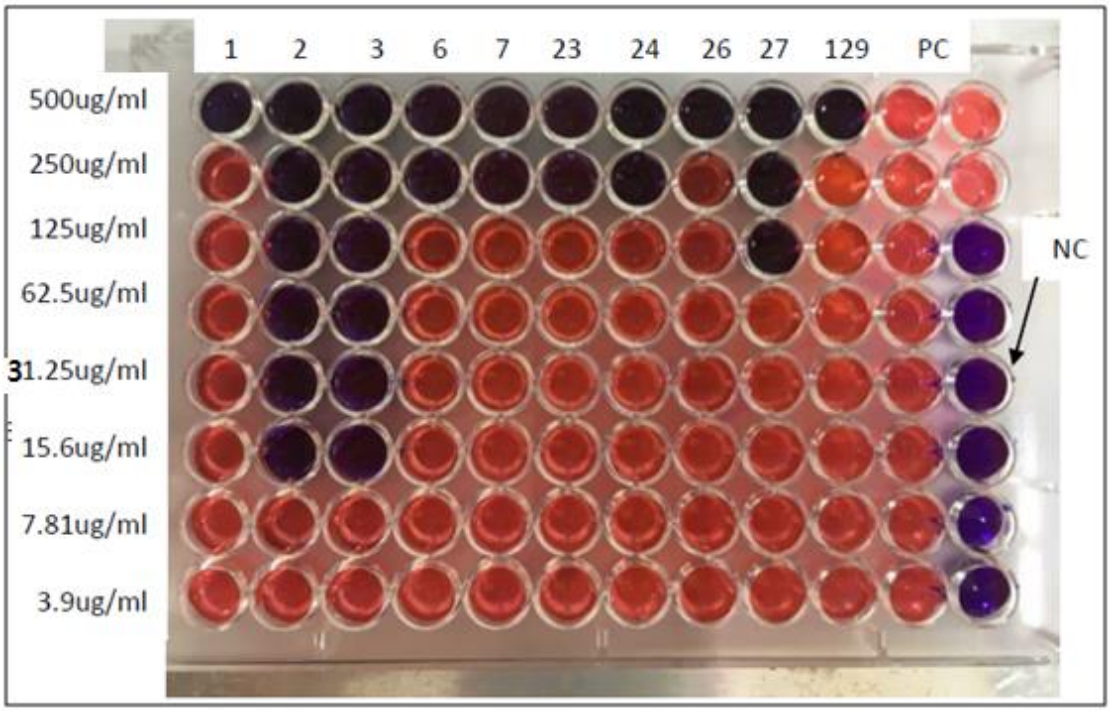

Figure 3. The minimum Inhibitory Concentrations (MICs) of Berberine at the concentrations (3.9-500 µg/ml) against *Klebsiella pneumoniae* Isolates by Microtiter Plate Assay with Resazurin Dye.

Discussion

In agreement with the current study, [26] indicated the Hormesis Effect of Berberine against Klebsiella pneumoniae and found that the presence of Berberine at low concentrations (25 and 50 µg/ml) resulted in higher minimal inhibitory concentrations of efflux-related antibiotics such as rifampicin and Azithromycin. Also, it was found that the potential risk of its applications against Gram-negative pathogens at low concentrations. Berberine (BEB), a natural isoquinoline alkaloid, is prevalent in numerous medicinal plants and has been

demonstrated to have antibacterial and antifungal activities alone or in combination with other drugs [27].

Berberine has a synergistic effect by enhancing the bacterial inhibition of some antibiotics [21]. One of the previous studies revealed that Berberine affects membrane integrity by altering the fatty acids contents in both saturated and unsaturated fatty acids and disrupting MRSA cell surface dose-dependently [21]. Plants are rich sources of valuable secondary metabolites, such as alkaloids, quinones, tannins, terpenoids, flavonoids, and polyphenols. Many studies focus on plant secondary metabolites as a potential source for antibiotic discovery. They have the required structural properties and can act by different mechanisms (Najwan et al., 2021). Several other studies evaluated the efficacy of both natural and synthetic EPIs on MDR strains of K. pneumoniae and other clinically significant nosocomial pathogens. They proved it efficacious in inducing sensitivity toward various classes of drugs Iman et al., 2018 and [24, 23].

Conclusion

Because the efflux pumps of bacteria actively contribute to the resistance to most antibiotics, using the potent Efflux pump inhibitors to target and block these pumps can help potentiate the old antibiotics effective against a range of drug-resistant bacteria. Efflux pump inhibitors such as Berberine are considered promising adjunctive therapeutic agents with known antibiotics to improve their antibacterial potency at low concentrations and reduce the emergence of antimicrobial resistance.

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation:Khalid, TWBM; Ghaima, K.K. Effect Berberine as efflux pump inhibitor in multidrug-resistant Klebsiella pneumoniae isolated from urinary tract infections. Revis Bionatura 2023;8 (2) 73. http://dx.doi.org/10.21931/RB/CSS/2023.08.02.73