Journal of Sciences, Islamic Republic of Iran 33(2): 117 - 124 (2022) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Evaluation of The Effect of Plantago Ovata Plant Extract on the Expression of Emerging Beta-lactamase Genes in Clinically Isolated Multidrug-resistant *Klebsiella pneumoniae* Strains of COVID-19 Patients

M. Rezaeizadeh[1], K. Amini[2*]

[1] *Department of Microbial biotechnology, Faculty of Biological Sciences, Tehran North Branch, Islamic Azad University, Tehran, Islamic Republic of Iran*
[2] *Department of Microbiology, Faculty of Sciences, Saveh Branch, Islamic Azad University, Saveh, Islamic Republic of Iran*

Received: 31 January 2022 / Revised: 8 June 2022 / Accepted: 20 July 2022

Abstract

This study aimed to investigate the effects of Plantago ovata plant extract on the expression of beta-lactamase-producing genes in multidrug-resistant (MDR) *K. pneumoniae* isolates. This study was conducted on 50 samples of COVID-19 patients admitted to the intensive care unit of respiratory hospitals. *K. pneumoniae* isolates were identified using standard biochemical tests, culturing, and Gram staining. The antibiotic susceptibility profile of isolates was determined using the micro broth dilution method. Then, P. ovata extract was prepared and its effects on the expression of MDR K. pneumoniae genes were evaluated. Totally, 50 samples were collected from 50 patients (25 males and 25 females, 58 ± 2.2 years of age) with COVID-19 infection. Thirty K. pneumoniae strains, 4 *K. oxytoca* strains, 2 K. mobilis strains, and 2 strains of *K. rhinoscleromatis* were isolated here. Gentamicin and chloramphenicol did not affect the strains and piperacillin/tazobactam was the most effective antibiotic. *CTX-M15, OXA-48*, and *OXA-181* genes were detected in 29 (96.6%), one (1.66%), and one (1.66%) *K. pneumoniae* strains, respectively. The minimum inhibitory concentration of P. Ovata was 3.125 µg/ml for the isolated bacteria, and the extract significantly downregulated *OXA-48* and *OXA-181* genes ($p<0.005$, CI=95%). P. ovata extract showed antibacterial effects on MDR species of clinically isolated *K. pneomoniae*. Downregulation of beta-lactamase enzyme-producing genes can be considered as the possible mechanism action of antibacterial effects of the plant.

Keywords: P. ovata; *K. pneomoniae*; Beta-lactamase; Antibacterial.

Introduction

Klebsiella pneumoniae is a non-motile, Gram-negative, lactose-fermenting encapsulated opportunistic pathogen that frequently causes urinary tract infections and pneumonia in immunocompromised individuals.

* Corresponding Author: Tel: +988642433342; Email: dr_kumarss_amini@yahoo.com

This bacterium, after *Escherichia coli*, is the most common cause of sepsis caused by Gram-negative bacteria and nosocomial infections [1-4]. Recent studies have shown that the global spread of multidrug-resistant (MDR) species of K. pneumoniae is of great concern. The Increasing use of antibiotics for therapeutic purposes in medicine and veterinary medicine, poultry, aquatic breeding, and agriculture, and the mechanisms of antibiotic resistance gene transfer have led to high selective pressure and thus the selection and spread of pathogenic MDR bacteria. "Klebsiella" species carry chromosomal and plasmid genes that play a major role in the virulence, resistance, and invasion of this microorganism [5]. Beta-lactamases are a family of hydrolytic enzymes that hydrolyze beta-lactam antibiotics to convert them into derivatives without antibacterial activity. Beta-lactamases are the main defense of Gram-negative bacteria against beta-lactam antibiotics [6]. By breaking down the beta-lactam ring in penicillin and cephalosporins, these enzymes inactivate them and protect the bacteria from the damaging effects of these drugs during the treatment process. Recently, new beta-lactamase enzymes, such as *OXA-48, OXA-181*, and *CTX-M-15*, have been identified in some bacteria, especially Klebsiella species, which cause bacterial resistance to many antibiotics such as penicillin's, carbapenems, aminoglycosides, macrolides, and sulfamethoxazole [7]. The gene that produces this enzyme is located on plasmids and can be transferred from one bacterium to another. Chromosomal beta-lactamases are located on specific species of bacteria, while plasmid beta-lactamases are more diverse and are transmitted between different species [8].

Studies have shown that genetic testing can increase the chances of treatment because some pathogens containing these genes may be mistakenly detected or not noticed in routine laboratory tests, and this can lead to the administration of ineffective antibiotics and spread and develop more resistant pathogens [9]. In addition, rising antibiotic resistance and side effects of chemical-based drugs have prompted scientists to use natural resources to replace these drugs [10]. Plantago ovata, also known as blond plantain, belongs to the Plantaginaceae family and is one of the most important native medicinal plants in Iran and various countries [11]. It has about 250 species, and compounds of benzoic acid, caffeic acid, fumaric acid, and ascorbic acid, and organic acids have been identified in its seeds. This genus has a global distribution and has long been used therapeutically in infections, as previously reported [12]. Today, the COVID-19 pandemic is the main issue of the health community that has led to the admission of many people to intensive care units (ICUs) around the world, with more than 5,000 ICU-admitted patients reported in August 2021 throughout Iran. As no definitive cure has yet been discovered for the disease, various treatment regimens are prescribed for patients, including immunosuppressive drugs, to prevent cytokine storm [1]. Weakening of the immune system with drugs, such as prednisolone and dexamethasone, as well as the use of ventilators can make patients more susceptible to infection with microorganisms. On the other hand, previous reports show that approximately 75% of COVID-19 patients receive broad-spectrum antibiotics. There are several reports of the prevalence of antibiotic resistance in these patients [4,3]. This study aimed to investigate the effects of P. ovata extract on emerging beta-lactamase genes in MDR *K. pneumoniae* strains isolated from clinical specimens of COVID-19 patients.

Materials and Methods

Isolation of Klebsiella strains

This study was conducted on samples of 50 patients admitted to ICUs of three respiratory hospitals (Shariati, Imam Khomeini and Masih Daneshvary) in Tehran municipal. These samples were collected from patients suspected of secondary bacterial infection and referred to the hospital laboratory. Bacteria were isolated and identified using cultures and standard biochemical analyses. The samples were first cultured on Hektoen enteric agar selective medium and incubated at 37 °C overnight. The media were then examined and yellow colonies suspected of Klebsiella species were cultured on TSI, urease, MRVP, arginine decarboxylase, lysine iron agar, ornithine decarboxylase, and Simon citrate mediums. Following confirming the existence of Klebsiella species using the methods determined by CLSI, their antibiotic susceptibility pattern was determined by the Kirby Bauer method [13].

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed for molecular typing of the isolated K. pneumoniae strains according to Elahi et al. [14]. Genomic DNA was extracted using a bacterial DNA extraction kit (QIAamp, Germany) and digested with 20U XbaI (Fermentas, Lithuania) restriction enzyme at 37 °C for 4 h. The fragments were then separated in 2% agarose gel using a CHEF-DRIII (Bio-Rad, USA), visualized under UV light after staining with Erythrogel, and the data were analyzed using Gelcompar II (Applied Maths, Belgium). PFGE dendrogram was generated using the MEGA 7 software with the unweighted pair-group method and the Dice coefficient.

Antibiotic susceptibility profile

The pattern of antibiotic susceptibility in Klebsiella strains was determined using a modified disk diffusion method against antibiotics [15]. Klebsiella species with turbidity following 0.5 McFarland standards were cultivated by a sterile cotton swab in Müller-Hinton agar (MHA) medium with 2% NaCl. Then, antibiotic discs (Padtan teb, Iran) were placed on the surface of the medium and incubated at 37 °C overnight. MDR strains were then determined according to the guidelines of the Centers for Disease Control and Prevention (CDC).

Multiplex PCR study

For molecular investigation and determining the presence of *CTX-M15, OXA-48*, and *OXA-181* genes in the strains, their whole genome was extracted using a special kit (QIAamp, Germany). The quantity and quality of extracted DNA were confirmed by a spectrophotometer (Nanodrop, Thermo Fisher Scientific, USA). The purity of DNA was determined by determining the optical density (OD) ratio at 260-280 nm (1.5-2), indicating proper purification of the DNA-containing solution. Following quality confirmation by Nanodrop (Eppendorf, Germany), specific primers for target genes were designed by Gene runner software and blasted on the NCBI website to confirm specificity (Table 1). The strains harboring *CTX-M15, OXA-48,* and *OXA-181* genes were detected by the multiplex-PCR test. PCR reaction was performed in a thermocycler (PEQ STAR, Germany) under the following conditions: initial denaturation step at 95 °C for 5 min and 35 cycles, including denaturation at 94°C for 30 seconds and primer binding at 55 °C for 30 seconds. The amplification step was performed at 72 °C for 1 minute; after 35 cycles, the final amplification step was carried out at 72 °C for 10 min. DNA fragments were separated by electrophoresis on 2% agarose gel in the presence of positive and negative controls and stained with GelRed® Nucleic Acid Gel Stain.

Preparation of P. ovata extract

Fresh P. ovata seeds were diagnosed by an expert in the realm of botany and collected in late spring from the mountains around Tehran (Iran). Plants were cleaned, washed, and dried in a dark and dry room. The dried seeds were pulverized by an electric mill. Then, 2000 g of the seed powder was mixed with 500 ml of distilled water and 96% ethyl alcohol (50:50), kept in the dark for 2 days, and shaken daily for 20 min. Erlenmeyer contents were then filtered through filter paper. The filtered liquid was extracted using a rotary evaporator at 50 °C in a vacuum and placed at 40 °C to evaporate water and alcohol. Different concentrations (10, 20, 40, and 80 mg/ml) of the hydroalcoholic extract of P. ovata were prepared in sterile distilled water.

Antimicrobial activity of the plant extract

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extract were determined against MDR K. pneomoniae isolates by the broth microdilution method in sterile plates. For this purpose, final bacterial numbers were standardized to 1×10^6 CFU/mL. Serial double dilutions of the P. ovata extract ranging from 10 to 80 mg/ml were poured in a 96-well microtiter plate containing 10 µL of Muller Hinton broth (MHB) medium, 10 µL of bacterial suspension was added to each well, and incubated at 37 °C for 24 h. Then, the density of bacteria in the liquid culture was determined using the OD measurement at 570 nm. MBC was determined by culturing a loop of a well with no bacterial growth in the tryptone soya agar. *Staphylococcus aureus* ATCC 25923 strains were used as the control.

Gene expression evaluation

To evaluate the effects of P. ovata extract on the expression of target genes using Real-time (RT)-PCR, MDR strains of K. pneumoniae were treated with a sub-MIC concentration of the extract for 24 h. Total bacterial RNA was extracted using an RNA isolation kit (QIAGEN, Germany) according to the manufacturer's

Table 1: Primers used to differentiate and isolate *Klebsiella* species harboring target genes

Gene	Primer sequence 5′ to 3′	Size (bp)	Gene bank accession number
CTX-M15	F = TCGTCTCTTCCAGAATAAGG R = AAGGGAACCAGGAACCACG	657	DQ302097
OXA-48	F = CCAAGCATTTTTACCCGCATCKACC R = GYTTGACCATACGCTGRCTGCG	438	NZ_CP018735
OXA-181	F = CCTAGATTCTACGTCAGTAC R = CTCTCTAGTCGGACAACACC	325	MN227183
16SrRNA	F = AGAGTTTGATCCTGGCTCAG R = GGTTACCTTGTTACGACTT	1500	KT257735

instructions and stored at −80 °C. Extracted RNA quality and quantity were evaluated using spectrophotometry and electrophoresis on the agarose gel. Then, cDNA was synthesized using Thermoscript reverse transcriptase (Invitrogen, USA), and RT-PCR was performed using a Real-time RT-PCR kit (QIAGEN, Germany) according to instructions. RT-PCR was performed on a StepOneplus System (Applied Biosystems, USA) using SYBR Green PCR Master MIX (Applied Biosystems, USA). Relative expression levels of *OXA-181, OXA-48*, and *CTX-M15* genes were quantified using the 16S rRNA gene as a reference. The specificity of the RT-PCR reaction was monitored with a melting curve analysis following the final step of the reaction.

Results

Isolation and typing of Klebsiella species

To conduct this study, sputum samples of patients with COVID-19 infections (25 males and 25 females, 58 ± 2.2 years of age) suspected to bacterial infection were examined. K. pneumoniae bacteria were isolated from the samples during different stages of isolation. Specimens were cultured and suspected colonies were stained in general and specific mediums. Out of 50 samples, 30 strains of *K. pneumoniae*, 4 strains of *K. oxytoca*, 2 strains of *K. mobilis*, and 2 strains of *K. rhinoscleromatis* were isolated after differential tests. The PFGE profile of isolates is delineated in Figure 1. According to the dendrogram, the PFGE image indicated that two strains (A5 and A6) had over 90% similarity.

Determining the pattern of antibiotic susceptibility

The pattern of antibiotic susceptibility of *K. pneumoniae* strains isolated from patients is shown in Figure 2. The data showed that the highest resistance occurred with gentamicin and chloramphenicol (100%), followed by cefepime and cefoperazone with the least effect on the strains. On the other hand, piperacillin-tazobactam had the greatest effect (76.5%) among all Multiplex PCR results.

The results of DNA and RNA analyses confirmed the quality and quantity of the extracted genome. The results of the Multiplex-PCR test to detect *K. pneumoniae* strains harboring *CTX-M15, OXA-48,* and *OXA-181* genes showed that 58 (96.6%), one (1.66%), and one (1.66%) strains carried these genes, respectively (Figure 3).

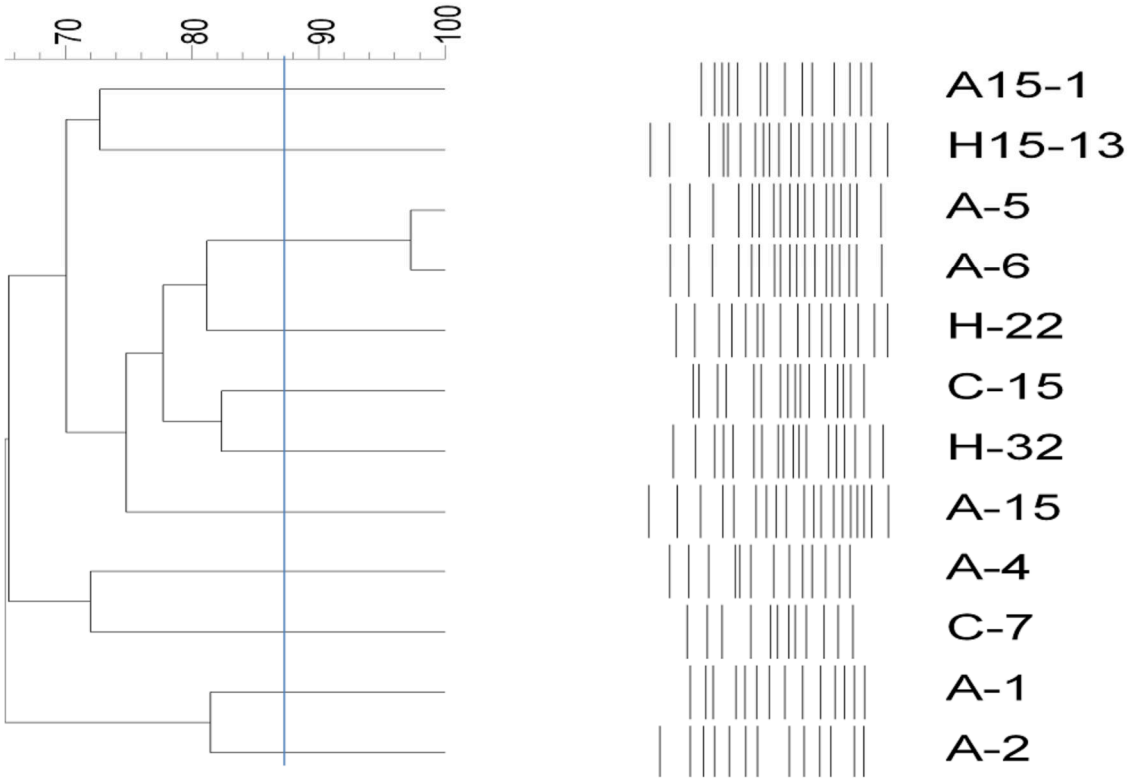

Figure 1. Dendrogram of the PFGE pattern for *K. pneomoniae* strains isolated in this study using PFGE data.

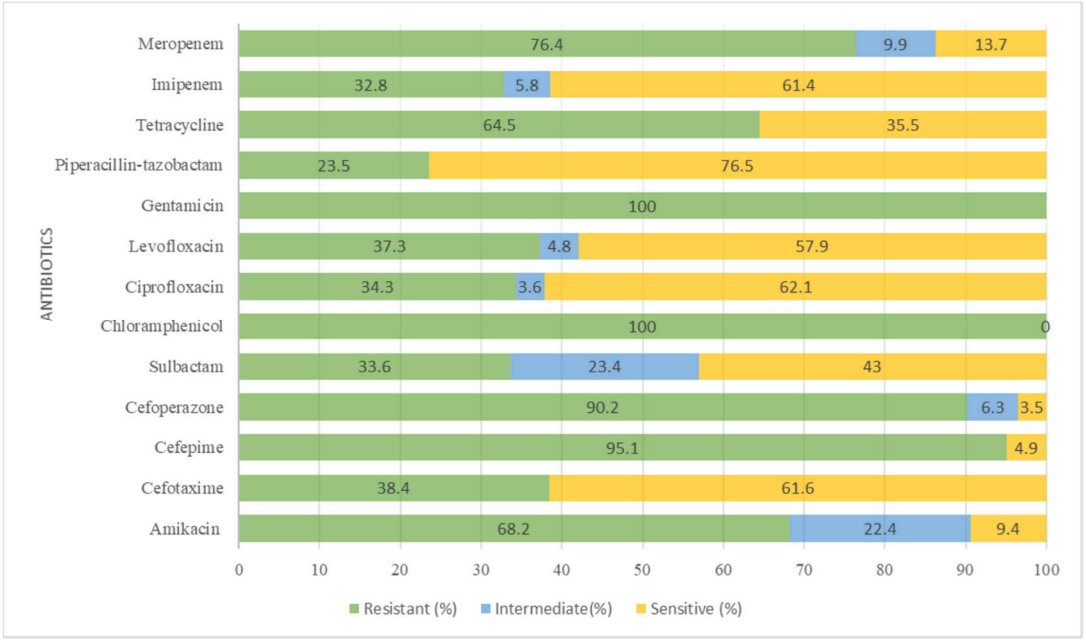

Figure 2. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from patient samples based on CLSI table

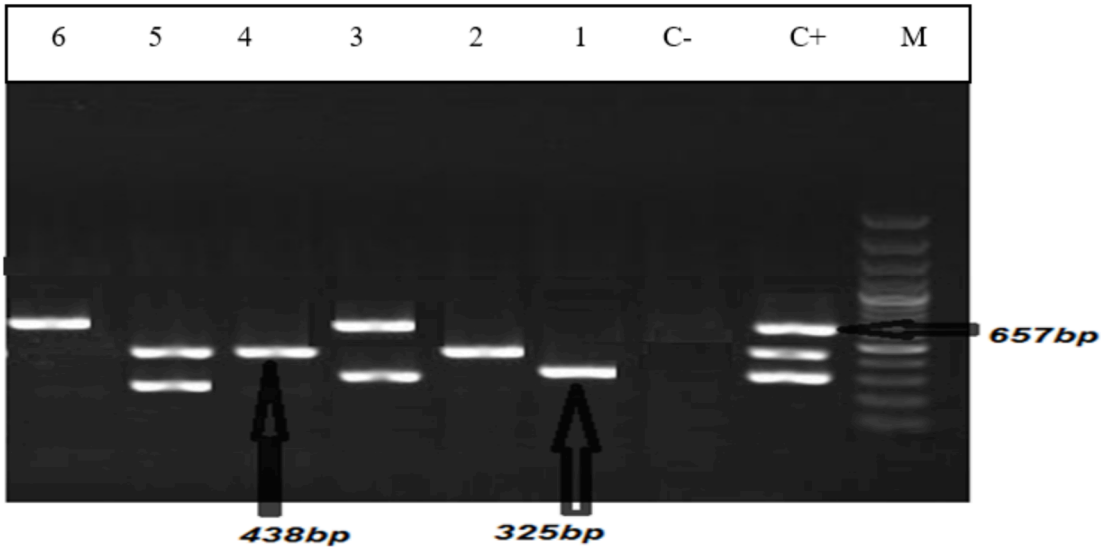

Figure 3. Electrophoresis of Multiplex PCR products of *Klebsiella pneumoniae* strains on 1% agarose gel. C: control, lanes 1-6: samples. M: 100 bp marke

MIC results of P. ovata extract

The MIC values for the P. ovata extract were determined for all MDR isolates by the microdilution broth method using concentrations of 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.4, 0.2, and 0.1 µg/ml. The results showed that the MIC for the isolated bacteria was 3.125 µg/ml while the sub-MIC and MBC levels were 1.56 and 6.25 µg/ml, respectively.

Expression patterns of genes

Expression patterns of the target and 16S rRNA genes were assessed after treatment of drug-resistant strains with the extract. Examination of the melting curve of RT-PCR reactions showed that these graphs were single-band and specific. The results of the t-test showed that the expression of *OXA-48* and *OXA-181* genes significantly decreased ($p < 0.005$, CI = 95%) after treatment with the P. ovata extract versus the

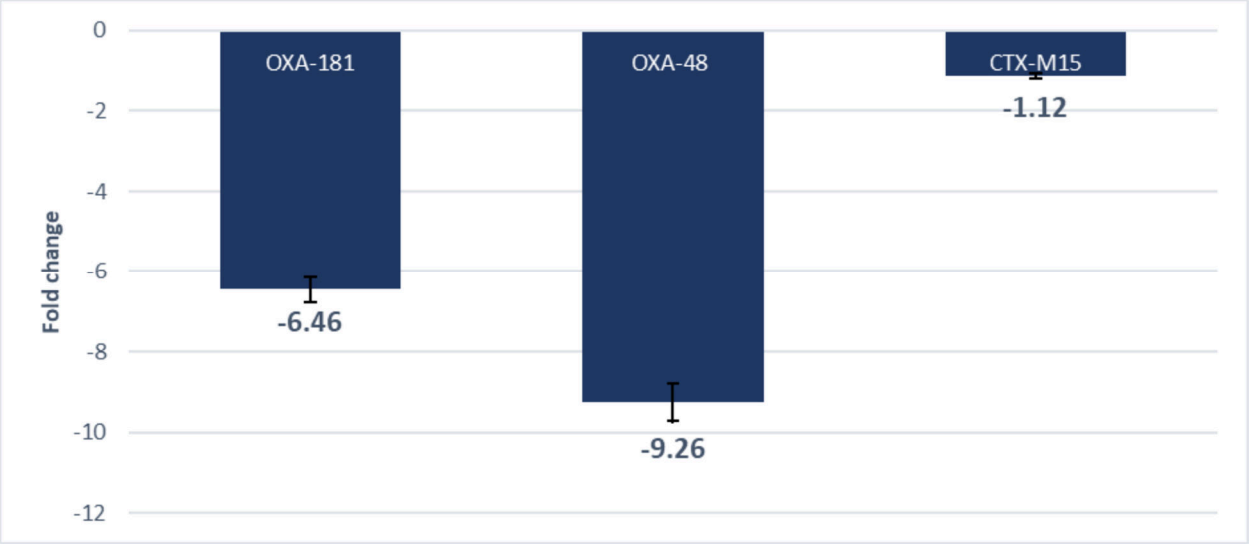

Figure 4. Fold change chart of target genes in multidrug-resistant strains of *Klebsiella pneumoniae* treated with the *P. ovata* extract versus the control

control (Figure 4). The data also showed that the CTX-m15 gene expression did not change significantly ($p > 0.065$).

Discussion

Antibiotic resistance is the ability of a microorganism to stop the effect of an antibiotic and is a major cause of failure in the treatment of infectious diseases, which increases the incidence of disease, mortality, and health care costs [16]. Studies have shown that resistance to beta-lactam drugs has increased alarmingly in K. pneumoniae strains, and plants are an important source of new antimicrobial drugs with different mechanisms than chemical antibiotics [17]. This study, therefore, investigated the effect of P. ovata extract, an old widely used medicinal plant, on MDR strains of K. pneumoniae in COVID-19 patients. It must be pointed out that secondary bacterial infections are difficult to diagnose because they can be masked by changes in the indicators of acute respiratory infection caused by COVID-19. Our data indicate that this plant has the bactericidal ability and can be used in the treatment of MDR strains of *K. pneumoniae* to reduce the expression of OXA-48 and OXA-181 genes. The present results of antibiotic resistance patterns showed that most of the isolated strains of *K. pneumoniae* were resistant to almost all antibiotics. Previous studies reported a direct relationship between the use of antibiotics and the emergence rate of MDR bacteria, and this resistance can be transmitted to other strains through plasmids [18]. Beta-lactams are the most widely used antimicrobial drugs, and beta-lactamases are the most important sources of antibiotic resistance [19]. This study showed acceptable rates of the efficacy of P. ovata extract on MDR isolates harboring new beta-lactamase enzymes. Motamedi et al. evaluated the effect of P. ovata extract against pathogenic bacteria and showed that the MIC of the extract was the same (20 mg/ml-1) against *S. aureus* and *Bordetella bronchiseptica* [20]. In contrast, Karami et al. showed that the extract P. ovata native to Iran had a moderate effect against Bacillus sphaericus and *Pseudomonas aeruginosa*, respectively, but did not affect MDR strains [21]. Our study showed bactericidal effects of P. ovata against MDR strains, which are reported in other studies [22, 23]. Numerous reports confirm the antibacterial effects of herbal medicines [24, 25], however it is first report about the effects of herbal medicines on beta lactamse genes expression. In line with these investigations, our study confirmed the antibacterial effects of P. ovata. The mechanism of this action is not revealed completely, but it has been attributed to the secondary metabolites of the plant that can harm bacterial cell walls. In this study, it has been shown that the extract can regulate the expression of the genes involved in beta-lactamase production. Tilili et al. evaluated the antibacterial activity and brine shrimp toxicity of leaf extracts from six Tunisian spontaneous species, including P. ovata, and detected no activity against Gram-negative bacteria [26]. This finding is in contrast with our study and this discrepancy can be attributed to the difference in the location of collected

plants, which can affect the extract compounds. Our data is in line with several studies on *K. pneomoniae* isolated from clinical sources in Iran [14, 27] [28] and other regions showing high phenotypic diversity and various genomic patterns among these strains [29, 30]. Studies by Tijet et al. [31], Dedeic-Ljubovic et al. [32], and Christian et al. [33] indicate fewer varieties of K. pneomoniae strains, which can be attributed to limited sources such as sampling from one medical center. The results of our study revealed the antibacterial effects of *P. ovata* extract on MDR species of clinically isolated *K. pneomoniae*. P. ovata leaves and seeds has been widely studied and it has been showed that they contain lipids, polysacharids (plantaglucid), alkaloids, flavonoids, caffeic acid derivatives, iridoid glycosides, vitamins and other organic elements preparing its medicinal assets [11, 12]. It should be mentioned that even though the CTX-M15 gene was observed in more than 96% of the strains, its gene expression was not significantly changed by the P.ovata extract. It seems that the mechanism of the antibacterial effect of P.ovata plant extract is based on the effect on OXA-48, and OXA-181 genes. This can be attributed to the difference in the sequence location of these genes in the bacterial genome. The antibacterial potential of this plant against various pathogens has been reported earlier, but the precise mechanisms of antibacterial action are not revealed according to the authors' knowledge. In this study, the plant extract showed an important effect on the downregulation of beta-lactamase-producing genes, that suggests it as a possible mechanism of action. Further evaluations are necessary for revealing the precise mechanism of action of the *P.ovata* extract.

References

1. Guisado-Gil AB, Infante-Domínguez C, Peñalva G, Praena J, Roca C, Navarro-Amuedo MD, et al. Impact of the COVID-19 pandemic on antimicrobial consumption and hospital-acquired candidemia and multidrug-resistant bloodstream infections. Antibiotics. 2020;9(11):816.
2. García-Meniño I, Forcelledo L, Rosete Y, García-Prieto E, Escudero D, Fernández J. Spread of OXA-48-producing Klebsiella pneumoniae among COVID-19-infected patients: The storm after the storm. J Infect Public Health. 2021;14(1):50-2.
3. Arteaga-Livias K, Pinzas-Acosta K, Perez-Abad L, Panduro-Correa V, Rabaan AA, Pecho-Silva S, et al. A multidrug-resistant Klebsiella pneumoniae outbreak in a Peruvian hospital: Another threat from the COVID-19 pandemic. Infect Control Hosp Epidemiol. 2021:1-2.
4. Wyres KL, Lam MM, Holt KE. Population genomics of Klebsiella pneumoniae. Nature Rev Microbiol. 2020;18(6):344-59.
5. Moradigaravand D, Martin V, Peacock SJ, Parkhill J. Evolution and epidemiology of multidrug-resistant Klebsiella pneumoniae in the United Kingdom and Ireland. MBio. 2017;8(1):e01976-16.
6. Mofolorunsho KC, Ocheni HO, Aminu RF, Omatola CA, Olowonibi OO. Prevalence and antimicrobial susceptibility of extended-spectrum beta lactamases-producing Escherichia coli and Klebsiella pneumoniae isolated in selected hospitals of Anyigba, Nigeria. African Health Sci. 2021;21(2):505-12.
7. Nakamura-Silva R, Oliveira-Silva M, Furlan JPR, Stehling EG, Miranda CES, Pitondo-Silva A. Characterization of multidrug-resistant and virulent Klebsiella pneumoniae strains belonging to the high-risk clonal group 258 (CG258) isolated from inpatients in northeastern Brazil. Arch Microbiol. 2021:1-9.
8. Projahn M, von Tippelskirch P, Semmler T, Guenther S, Alter T, Roesler U. Contamination of chicken meat with extended-spectrum beta-lactamase producing-Klebsiella pneumoniae and Escherichia coli during scalding and defeathering of broiler carcasses. Food Microbiol. 2019;77:185-91.
9. Liu C, Guo J. Hypervirulent Klebsiella pneumoniae (hypermucoviscous and aerobactin positive) infection over 6 years in the elderly in China: antimicrobial resistance patterns, molecular epidemiology and risk factor. Ann Clin Microbiol Antimicrob. 2019;18(1):1-11.
10. Khosravi M, Mirzaie A, Kashtali AB, Noorbazargan H. Antibacterial, anti-efflux, anti-biofilm, anti-slime (exopolysaccharide) production and urease inhibitory efficacies of novel synthesized gold nanoparticles coated Anthemis atropatana extract against multidrug-resistant Klebsiella pneumoniae strains. Arch Microbiol. 2020;202:2105-15.
11. Franco EAN, Sanches-Silva A, Ribeiro-Santos R, de Melo NR. Psyllium (Plantago ovata Forsk): From evidence of health benefits to its food application. Trends Food Sci Technol. 2020;96:166-75.
12. Phan JL, Cowley JM, Neumann KA, Herliana L, O'Donovan LA, Burton RA. The novel features of Plantago ovata seed mucilage accumulation, storage and release. Sci Rep. 2020;10(1):1-14.
13. Yin D, Guo Y, Li M, Wu W, Tang J, Liu Y, et al. Performance of VITEK 2, E-test, Kirby–Bauer disk diffusion, and modified Kirby–Bauer disk diffusion compared to reference broth microdilution for testing tigecycline susceptibility of carbapenem-resistant K. pneumoniae and A. baumannii in a multicenter study in China. Eur J Clinl Microbiol Infect Dis. 2021;40(6):1149-54.
14. Elahi A, Akya A, Chegene Lorestani R, Ghadiri K, Baakhshii S. Molecular Typing of Klebsiella pneumoniae Isolated from Medical Centers in Kermanshah Using Pulse Field Gel Electrophoresis. Arch Pediatr Infect Dis. 2019;7(2).
15. Yadav NS, Sharma S, Chaudhary DK, Panthi P, Pokhrel P, Shrestha A, et al. Bacteriological profile of neonatal sepsis and antibiotic susceptibility pattern of isolates admitted at Kanti Children's Hospital, Kathmandu, Nepal. BMC Res Notes. 2018;11(1):1-6.
16. Yelin I, Kishony R. Antibiotic resistance. Cell. 2018;172(5):1136-. e1.

17.Montiel-Riquelme F, Calatrava-Hernández E, Gutiérrez-Soto M, Expósito-Ruiz M, Navarro-Marí JM, Gutiérrez-Fernández J. Clinical Relevance of Antibiotic Susceptibility Profiles for Screening Gram-negative Microorganisms Resistant to Beta-Lactam Antibiotics. Microorganisms. 2020;8(10):1555.
18.Shin S, Jeong SH, Lee H, Hong JS, Park M-J, Song W. Emergence of multidrug-resistant Providencia rettgeri isolates co-producing NDM-1 carbapenemase and PER-1 extended-spectrum β-lactamase causing a first outbreak in Korea. Ann Clin Microbiol Antimicrob. 2018;17(1):1-6.
19.Banik BK. Beta-lactams: Novel synthetic pathways and applications: Springer; 2017.
20.Motamedi H, Darabpour E, Gholipour M, Seyyednejad S. Antibacterial effect of ethanolic and methanolic extracts of Plantago ovata and Oliveria decumbens endemic in Iran against some pathogenic bacteria. Int J Pharmacol. 2010;6(2):117-22.
21.Karami L, Ghahtan N, Habibi H. Antibacterial effect of plantago ovata and lallemantia iberica seed extracts against some bacteria. Res Mol Med. 2017;5(3):32-6.
22.Seyyednejad S, Motamedi H. A review on native medicinal plants in Khuzestan, Iran with antibacterial properties. Int J Pharmacol. 2010;6(5):551-60.
23.Sharma A, Verma R, Ramteke P. Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens. World Appl Sci J. 2009;7(3):332-9.
24.Hassan G, Ghafoor S. Herbal Medicines: An Adjunct to Current Treatment Modalities for Periodontal Diseases. Biomedica. 2020;36(1).
25.Zhu F. A review on the application of herbal medicines in the disease control of aquatic animals. Aquaculture. 2020;526:735422.
26.Tlili H, Marino A, Ginestra G, Cacciola F, Mondello L, Miceli N, et al. Polyphenolic profile, antibacterial activity and brine shrimp toxicity of leaf extracts from six Tunisian spontaneous species. Nat Prod Res. 2021;35(6):1057-63.
27.Ghotaslou R, Ghorashi Z, Nahaei M. Klebsiella pneumoniae In neonatal sepsis: a 3-year-study in the pediatric hospital of Tabriz Iran. Jpn J Infect Dis. 2007;60(2/3):126.
28.Heidary M, Nasiri MJ, Dabiri H, Tarashi S. Prevalence of drug-resistant Klebsiella pneumoniae in Iran: a review article. Iran J Public Health. 2018;47(3):317.
29.Gona F, Comandatore F, Battaglia S, Piazza A, Trovato A, Lorenzin G, et al. Comparison of core-genome MLST, coreSNP and PFGE methods for Klebsiella pneumoniae cluster analysis. Microb Genom. 2020;6(4).
30.Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, et al. Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother. 2009;53(8):3365-70.
31.Tijet N, Sheth PM, Lastovetska O, Chung C, Patel SN, Melano RG. Molecular characterization of Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae in Ontario, Canada, 2008-2011. PLoS One. 2014;9(12):e116421.
32.Dedeic-Ljubovic A, Hukic M, Pfeifer Y, Witte W, Padilla E, López-Ramis I, et al. Emergence of CTX-M-15 extended-spectrum β-lactamase-producing Klebsiella pneumoniae isolates in Bosnia and Herzegovina. Clin Microbiol Infect. 2010;16(2):152-6.
33.Christian NA, Roye-Green K, Smikle M. Molecular epidemiology of multidrug resistant extended spectrum beta-lactamase producing Klebsiella pneumoniae at a Jamaican hospital, 2000-2004. BMC Microbiol. 2010;10(1):1-8.