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In silico synergistic effects of polyhydroxyalkonates with antibiotic resistance profile of *Klebsiella pneumoniae* as promising way in pneumonia treatment

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ABSTRACT

Klebsiella pneumoniae is an important gram-negative opportunistic pathogen that causes a variety of infectious diseases, including urinary tract infections, bacteremia, pneumonia, and liver abscesses. In this study, *Klebsiella pneumoniae* strain KP2211 was isolated, identified, and subjected to in vitro assessment of its antibiotic sensitivity testes, and *in silico* predicted synergistic combination between antibiotic resistance profile with retrative polyhydroxyalkanoates compounds against important receptor protein play important role in multi-drug resistance treatment in Gram negative bacteria. Polyhydroxyalkonates (PHAs) are a class of naturally occurring, biodegradable polymers synthesized by various microorganisms as intracellular energy storage materials under conditions of nutrient imbalance. These polymers are composed of hydroxyalkanoate monomers and are characterized by their biocompatibility, biodegradability, and versatile material properties, making them suitable for a wide range of applications in biomedical fields (e.g., drug delivery, tissue engineering, and medical implants) and environmental sectors (e.g., biodegradable plastics, packaging, and agricultural films). The antibiotic susceptibility test reported that *klebsiella pneumoniae* strain KP2211 showed the highest resistance against different antibiotics as Amoxycillin (AMX), Cefepime (FEP), Vancomycin (VA), Cefoxitin (FOX), Ceftazidime (CAZ), Cefazoline (CZ), Trimethoprim/Sulfamethoxazole (SXT) and Clindamycin (CD). In addition, the prediction of a synergistic effect of PHAs ingredients as 9,12-Octadecadienoic acid (z, z) methyl ester and resistance antibiotic against LpxC enzyme in the lipid A biosynthetic pathway, showed a promising binding affinity ranging from -6.9 to -9.0 kcal/ mol. Our findings indicated that predicted molecules are a promising way to be used as a drug delivery system for multi-drug resistance in pneumonia treatment.

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Introduction

Klebsiella pneumoniae (K. pneumoniae) is a gram-negative bacterium predominantly located in the respiratory tract and intestines of humans, frequently responsible for nosocomial infections, resulting in conditions such as pneumonia, liver abscesses, soft tissue infections, urinary tract infections, bacteremia, and, in certain instances, mortality (Bengoechea & Pessoa 2019). The imprudent use of certain antibiotics and bacterial evolution have rendered drug-resistant diseases-a

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significant public health concern, in 2019, almost 1.2 million individuals succumbed to drug-resistant bacterial illnesses, surpassing the fatalities attributed to HIV/AIDS or malaria. *K. pneumoniae* is one of the six principal drug-resistant bacteria (Murray et al., 2022; Li et al., 2024).

The World Health Organization acknowledges that extended-spectrum β-lactamase (ESBL) and carbapenem-resistant *Klebsiella pneumoniae* (CRKP) present substantial public health risks. The global resistance rate of multidrug-resistant bacteria is rising, while the detection rate of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) in the United States has escalated from below 0.1% to 24.6%.

The resistance rate of *K. pneumoniae* isolates in Greek hospital wards is 52.4%. The China Antibiotic Surveillance Network (CHINET) indicates that the resistance rate of *K. pneumoniae* to carbapenems rose from 2.4% to 32.8% (Hu et al., 2022). The growing resistance has severely limited therapy choices and makes treating infections with multidrug-resistant (MDR) and exceptionally drug-resistant (XDR) *K. pneumoniae* a global issue (Li et al., 2023).

The search for novel therapeutic approaches has become very pressing. *K. pneumoniae* presents an imminent danger to humans and is progressively acquiring multi-resistance as the disease evolves. Nevertheless, there were no sanctioned medicines or vaccinations aside from conventional antibiotics, and there was a need to expedite the discovery of novel antibiotics and investigate alternative therapeutic strategies.

Numerous research has concentrated on identifying medicines that exhibit synergistic effects with polymyxins, including sertraline, levopromazine, the lipid membrane enzyme (LpxC) inhibitor was identified to be a potential and attractive target for antibiotic development as Chir-090, and selective estrogen receptor modulators (Hussein et al., 2017; Otto et al., 2019). Polyhydroxyalkanoates (PHAs) are biodegradable, low-cost, and ecofriendly polymers produced by various bacteria in the environment and PHAs compounds, including hexadecenoic acid and octadecanoic acid acts as drugs carriers used against liver hepatocellular carcinoma (HCC) (Hamedo et al. 2025).

The objective of this study was to predict the *in-silico* synergistic effects of polyhydroxyalkanoates on the antibiotic resistance profile of *K. pneumoniae* as a promising approach for pneumonia treatment and inhibition of target proteins in multidrug resistant Gram-negative bacteria.

Materials and Methods

Sampling

A sputum sample collected from Arish General Hospital (AGH) in North Sinai Governorate from a 30-year-old patient with suspected pneumonia and sample was transported to the laboratory and processed within 2 hours of collection.

Isolation and biochemical identification of Klebsiella pneumonia

Streak plate method was used for isolation of *K. pneumoniae* from the collected human sample. The sample were streaked on MacConkey agar plates (a selective and differential medium for Gram-negative bacteria, and blood agar (an enriched medium used for cultivating fastidious organisms and differentiating bacteria based on hemolytic properties, then incubated at 37°C for 24 h. The isolate was examined for presence of lactose fermented *K. pneumoniae* colony on MacConkey agar and on blood agar for detection of hemolytic activity (Manual 1998).

The suspected colonies were carefully selected and sub-cultured onto sterile nutrient agar, followed by incubation at 37°C for 24 hours. After incubation, the colonies were examined for their cultural characteristics and subjected to microscopic analysis using Gram staining to assess their morphological features (Smith & Hussey, 2005). A single colony exhibiting typical colonial morphology and consistent microscopic characteristics was chosen and propagated in semi-solid nutrient agar tubes for further identification (MacConkey 1905).

For the biochemical characterization of *Klebsiella pneumonia*, the suspected colonies were subjected to the following biochemical tests include carbohydrate fermentation (glucose and lactose) (Buckel & Barker 1974). citrate utilization (Wauters & Vaneechoutte 2015), catalase production (Cappuccino & Sherman 2014) and the Motility-Indole-Ornithine (MIO) test according to Ederer and Clark (1970). Additionally, the isolate was tested for urease production (Christensen, 1946).

Molecular identification of Klebsiella pneumonia

Bacterial genomic DNA was extracted using the PrepMan® Ultra Sample Preparation Reagent Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Briefly, 1 mL of bacterial broth culture was centrifuged at 13,000 rpm for 2 minutes, and the supernatant was discarded. The cell pellet was resuspended in 100 µL of PrepMan Ultra reagent, vortexed vigorously for 10–30 seconds, and heated at 100°C for 10 minutes in a water bath. After

cooling to room temperature, the sample was centrifuged at 13,000 rpm for 2 minutes, and 50 µL of the supernatant was collected and stored at -20°C for further use. For PCR amplification of the 16S rRNA gene was amplified by Using a set of universal primers (Invitrogen, USA), for bacteria as forward 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3'and reverse 1492R 5' (TAC GGY TAC CTT GTT ACG ACT)3' primers (Elshafey et al., 2022). PCR cycling conditions included an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 2 minutes, and extension at 68°C for 1.5 minutes, with a final extension at 68°C for 10 minutes and a hold at 4°C. We prepared the samples in accordance with Macro Gen Company's instructions, using 50 ng/l of PCR product. The samples were transported to Macro Gen Company in Korea, in which they were processed. Preliminary examination of sequences was performed using BLAST (http://www.ncbi.nlm/NLM.gov/BLAST) and cluster analysis was carried out using MEGA X software package (Badawi et al. 2024).

Antibiotic sensitivity test

To determine the susceptibility of *K. pneumoniae* to various commercial antibiotics, the Kirby-Bauer disk diffusion method (Bauer et al. 1966; Humphries et al. 2021). As recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines, fresh pure colonies from each isolate were suspended in 5 mL of 0.9% normal saline, and the optical density (OD) was adjusted to 0.5 OD. A sterile cotton swab was dipped into the bacterial suspension and streaked evenly over Mueller-Hinton agar (MHA) plates. After drying, standardized antibiotic disks (HiMedia) containing specific concentrations of selected antibiotics were placed on the agar surface using sterile forceps, ensuring proper contact and spacing (20 mm from the edge and 40 mm between disks). The plates were left at room temperature for 15 minutes to allow diffusion, then inverted and incubated at 37±2°C for 16–18 hours. Following incubation, the zones of inhibition were measured in millimeters and interpreted as susceptible (S), intermediate (I), or resistant (R) based on CLSI breakpoints. Isolates resistant to three or more antibiotic classes were classified as multidrug-resistant (MDR). The following antibiotics set were used in this study: Amoxycillin (10µg); Imipenem (30µg); Levofloxacin (5µg); Doxycycline (30µg); Cefepime (30µg); Amikacin (30µg); Azithromycin (15µg);Vancomycin (30µg); Cefoxitin (30µg); Ceftazidime (30µg); Chloramphenicol (30µg); Cefazoline (30µg); Gentamycin (10µg); Trimethoprim/Sulfamethoxazole (1.25/23.75µg), and Clindamycin (2µg) These commercially standardized paper disks were provided by HiMedia Laboratories. The diameter of the inhibition zone around each antibiotic disk was interpreted according to the national committee for clinical laboratory standards Institute (CLSI 2020).

Polyhydroxybutyrate synergy with Klebsiella pneumonia antibiotic resistance in silico

Ligand preparation

Polyhydroxyalkanoates was acted as drug delivery systems through the treatment process for various disease according to Hamedo et al. (2025), the biodegradable polyhydroxyalkanoates biopolymer produced by moderate halophilic bacteria consisting of promising carrier for drugs as Octadecadienoic acid which its chemical formula is $C_{19}H_{34}O_2$ was retrieved from database and some resistance antibiotic profile for *K. pneumoniae* reported its chemical formula represented in Table (1).

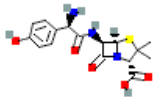
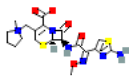
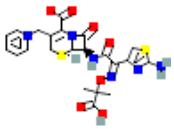
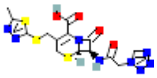
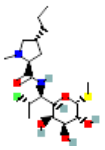
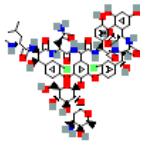
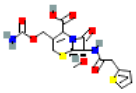
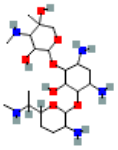
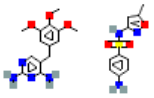
Forward reaction prediction of the synergistic combination between polyhydroxyalkanoates compounds and antibiotics.

A set of polyhydroxyalkanoates compounds have been obtained from database for docking purpose, 6 compounds have been collected from the PubChem database (Table1), and the predicted compound resulting from the synergistic combination between PHAs and antibiotics using (https://askcos.mit.edu/) was prepared for docking analysis using Avogadro 1.2.0 software. The 2D structure of ligand complexes was modeled using BIOVIA Discovery Studio 2021. Molecular docking was conducted using AutoDockTools_1.5.7_vina docking approach. The drug-likeness of PHAs compounds was then assessed using Lipinski's rule of five (Elshafey et al. 2025).

Protein preparation

The LpxC enzyme in the lipid A biosynthetic pathway was one of the most promising and clinically unexploited antibiotic targets for treatment of multidrug-resistant Gram-negative infections. Progress in medicinal chemistry had led to the discovery of potent LpxC inhibitors with a variety of chemical scaffolds and distinct antibiotic profiles (lee et al., 2014), and X-ray crystallographic structure of E. coli LpxC (EcLpxC) (PDB Id: 4MQY, Chain A) (Figure 1), was identified as the most suitable template for modelling K. pneumoniae HS11286 LpxC (KpLpxC) using MODELLERv.9.12 and other web-based software (Fiser, 2010).

Table 1 The chemical structure of some antibiotic resistance profile of *Klebsiella pneumonia*

Antibiotics	Chemical formula	Chemical structure
Amoxicillin	$C_{16}H_{19}N_3O_5S$	
Cefepime	$C_{19}H_{24}N_6O_5S_2$	
Ceftazidime	$C_{22}H_{22}N_6O_7S_2$	
Cefazoline	$C_{14}H_{14}N_8O_4S_3$	
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	
Vancomycin	$C_{66}H_{75}Cl_2N_9O_{24}$	
Cefoxitin	$C_{16}H_{17}N_3O_7S_2$	
Gentamycin	$C_{21}H_{43}N_5O_7$	
Trimethoprim/Sulfamethoxazole	$C_{24}H_{29}N_7O_6S$	

The existing research indicates that this protein was crucial for the treatment of multidrug-resistant Gram-negative infections. PDB structure of respective protein was collected from RCSB Protein Data Bank (rscsb.org). BIOVIA Discovery Studio 16.1 was used to clean and prepare all of the protein structures by taking out the water molecules and complex co-structures (Cole et al., 2011; Lee et al., 2011). Using Auto-Dock Tools version 1.5.6, polar hydrogens were included, and non-polar hydrogens were combined. To determine the binding interactions and binding affinities between the identified metabolites and previously chosen proteins, docking was performed using Auto Dock Vina (version 1.1.2) (Hamedo et al., 2025).

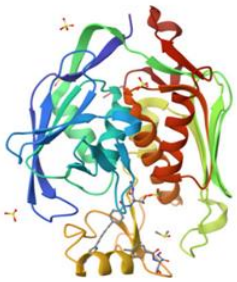


Fig 1. 3D structure of the lipid membrane enzyme (LpxC) protein with resolution 2.00 Å

Results

Isolation and biochemical characterization of Klebsiella pneumoniae strain KP2211

Out of 30 possible *Klebsiella pneumoniae* isolates that were previously identified by biochemical and culture techniques, the bacterial isolate exhibited Gram-negative rods under microscopic examination and was confirmed to be non-spore-forming. Culturally, the isolate formed pink colonies on MacConkey's agar, indicative of lactose fermentation, and circular, raised, entire, and white colonies on nutrient agar. No hemolysis was observed on blood agar, confirming its inability to hydrolyze red blood cells. Biochemically, the isolate demonstrated the ability to ferment both glucose and lactose, utilize citrate, and produce catalase. While the Motility-Indole-Ornithine (MIO) test was negative for indole production, ornithine decarboxylation, and motility but positive for urease production.

DNA extraction and molecular conformation of Klebsiella pneumoniae

DNA extraction from *Klebsiella pneumoniae* was used for molecular confirmation. And according to our pervious study conducted by (Badawi et al., 2024) the accession number for it in the GenBank database is OQ927599.1. Besides the phylogenetic analysis of the 16S rRNA gene sequences, this strain clustered with members of the *Klebsiella* genus with 99 % similarity (figure 2).

Antibiotic susceptibility test against Klebsiella pneumoniae strain KP2211

The result revealed that *Klebsiella pneumoniae* strain KP2211 showed the highest resistance against different antibiotics such as Amoxycillin (AMX), Cefepime (FEP), Vancomycin (VA), Cefoxitin (FOX), Ceftazidime (CAZ), Cefazoline (CZ), Trimethoprim/ Sulfamethoxazole (SXT), Clindamycin (CD) (figure 3).

Forward reaction prediction of the synergistic combination between polyhydroxyalkanoates and Antibiotics resistance to Klebsiella pneumoniae strain KP2211

In this investigation, *in silico* analysis of the predicted compounds derived from the synergistic combination between polyhydroxyalkanoates and Antibiotics resistance to *Klebsiella pneumoniae* strain KP2211 displayed the potential to inhibit The LpxC enzyme in the lipid A biosynthetic pathway was one of the most promising and clinically unexploited antibiotic targets for treatment of multidrug-resistant Gram-negative infections. The binding energies between predicted compounds and amino acids of receptor proteins linked to LpxC enzyme are shown in table 2.

While predicted compounds resulting from combination between 9,12-Octadecadienoic acid (z,z) methyl ester and Amoxycillin (AMX) showed high binding energy with receptor protein LpxC enzyme -8.6 Kcal/ mol. This study identified conventional hydrogen bonds with methionine **(**MET) A:61, leucine **(**LEU) A:62. Nevertheless, PI-sulfur interactions were noted with cysteine **(**CYS) A:63, histidine (HIS) A:238 which may contribute to the effectiveness in suppressing the activity of LpxC receptor (figure 4A).

Also predicted compounds resulting from combination between 9,12-Octadecadienoic acid (z,z) methyl ester and Cefazoline displayed good binding affinity of -8.7 Kcal/ mol with receptor protein LpxC enzyme, due to the presence of PI-sulfur interactions were noted with CYS A:63, HIS A:238 beside conventional hydrogen bond with amino acids residues (figure 4B). Furthermore, the predicted compounds arising from combination between 9,12-Octadecadienoic acid (z,z) methyl ester and Cefepime displayed higher binding affinity of -9.0 Kcal/ mol with receptor protein LpxC enzyme, due to the presence of PI-sulfur and pi cation interaction in amino acids CYS A:63, HIS A:238, HIS A:268, **(Figure 4C)**. Meanwhile, the predicted compounds arising from combination between 9,12-Octadecadienoic acid (z,z) methyl ester and clindamycin showed binding affinity of -6.9 Kcal/ mol with receptor protein LpxC enzyme, may be due to the presence of unfavorable Aceptor-Aceptor interactions in amino acids LEU A:62 as shown in (Figure 4D).

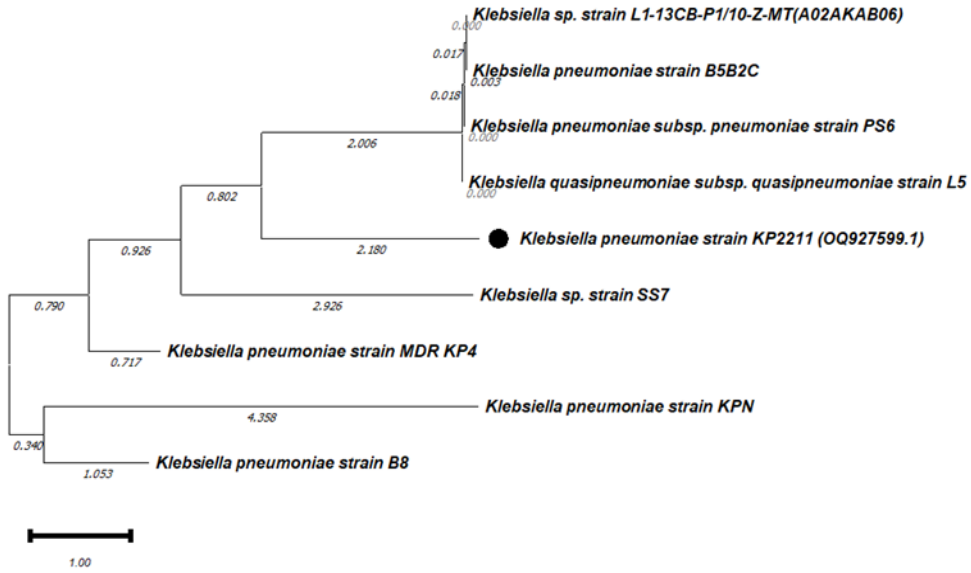


Fig 2. Phylogenetic tree of *Klebsiella pneumoniae strain* KP2211 using Neighbor-Joining method.

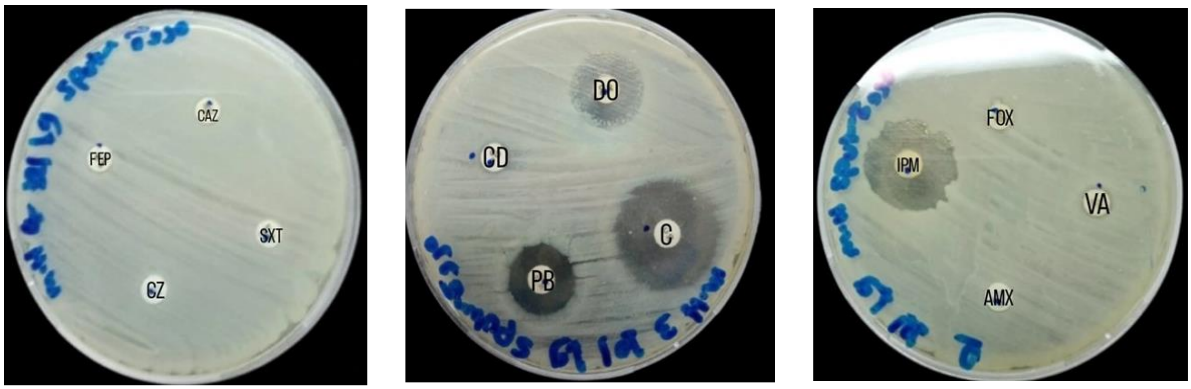


Fig 3. Antibiotic profiles of *Klebsiella pneumoniae* strain KP2211.

Table 2 Molecular docking analysis of the predicted compounds resulting from the synergistic combination between polyhydroxyalkanoates (PHAs) biopolymer and some resistance antibiotic profile

Forward reaction prediction of compounds	Target protein for treatment of multidrug-resistant Gram-negative infections	Binding affinity Kcal/mol
9,12-Octadecadienoic acid (z,z) methyl ester and Amoxycillin (AMX)	LpxC enzyme in the lipid A biosynthetic pathway	-8.6
9,12-Octadecadienoic acid (z,z) methyl ester and Cefepime (FEP)		-9.0
9,12-Octadecadienoic acid (z,z) methyl ester Ceftazidime (CAZ)		-8.5
9,12-Octadecadienoic acid (z,z) methyl ester Cefazoline (CZ)		-8.7
9,12-Octadecadienoic acid (z,z) methyl ester and Clindamycin (CD)		-6.9

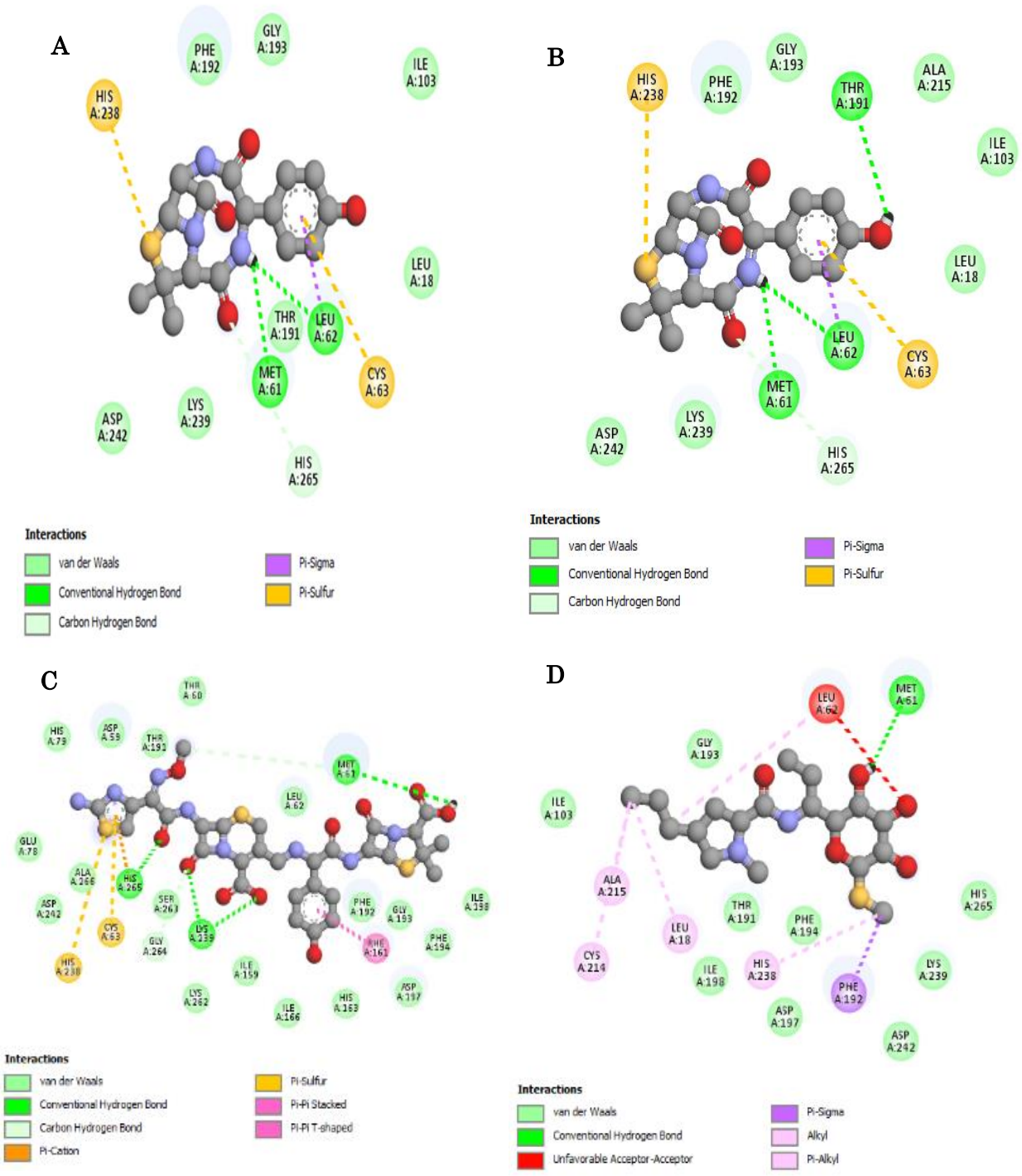


Fig 4. Molecular docking interactions between predicted compounds from the synergistic combination between the most potent polyhydroxyalkanoates (PHAs) ingredients and antibiotic resistance to *Klebsiella pneumoniae* strain KP2211. A: Predicted compound from 9,12-Octadecadienoic acid (z,z) methyl ester and Amoxycillin (AMX), B: Predicted compound from 9,12-Octadecadienoic acid (z,z) methyl ester and Cefazoline (CZ), C: Predicted compound from 9,12-Octadecadienoic acid (z,z) methyl ester and Cefepime (FEP) and D: Predicted compound from 9,12-Octadecadienoic acid (z,z) methyl ester and Clindamycin(CD)

Discussion

Klebsiella pneumoniae is a class of gram-negative bacterium that is ubiquitously found on the surface of mucosa in animals, or in the environment (such as water, soil, etc.). In humans, *K. pneumoniae* was concentrated in the gastrointestinal tract, and a few in the nasopharynx, through which the bacteria can enter the blood circulation or other tissues and then cause infection. In the era of pre-antibiotics, *K. pneumoniae* was a vital pathogen of community-acquired pneumonia (CAP), especially in diabetics and alcoholics. In the era of antibiotics that followed, it became a major cause of medical-related infections in hospitals (Podschun & Ullmann, 1998) and a risk factor of severe community-acquired infections (Holt et al., 2015). Characteristic of many members of the Enterobacteriaceae family, the microscopic analysis of the bacterial isolate in this work revealed Gram-negative, rod-shaped non-spore-forming cells (Moxley, 2022). The Gram-negative character of the bacterium points to the existence of an outer membrane with lipopolysaccharides, which in some bacterial species can cause pathogenicity and antibiotic resistance (Huszczynski et al. 2019).

Cultural traits helped to define isolation even further. Common in coliform bacteria including *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp., pink colonies on MacConkey's agar confirmed their capacity to ferment lactose (Kaluba, 2024). Typical of many environmental and clinical isolates, the circular, elevated, whole white colonies grown on nutrient agar showed non-pigmented bacterial growth. Furthermore, the lack of hemolysis on blood agar indicated that the bacterium differs from pathogenic hemolytic bacteria such as *Streptococcus pyogenes* and *Staphylococcus aureus* in that it lacks hemolysins capable of lysing red blood cells. Further understanding of the isolate's metabolic capacity came from the biochemical profile. Consistent with lactose-fermenting Enterobacteriaceae, the capacity to ferment both glucose and lactose was the positive catalase test found the catalase enzyme, which detoxifies hydrogen peroxide—a feature of aerobic and facultative anaerobic bacteria (Hepşen et al., 2024). Furthermore, the capacity to use citrate as a solitary carbon source points to citrate-permease, therefore enabling survival in nutrient-limited conditions (Kaluba, 2024).

The MIO test's findings offered still more distinct separation. Different from *Escherichia coli*, a lactose-fermenting, indole-positive bacteria, the negative indole test implies the absence of tryptophanase (Forbes et al., 2016). Unlike highly motile bacteria like *Proteus* spp., the negative motility test indicated the bacterium lacks flagella, therefore differentiating it from others. The negative ornithine decarboxylation test revealed that an inability to use ornithine as an energy source, so further restricting its identification. But the positive urease test indicated that urea may be broken down into ammonia and carbon dioxide, a feature usually linked to *Klebsiella* and *Proteus* species (Akash, 2024).

Rising frequency of multidrug-resistant (MDR) Gram-negative bacterial infections gravely affected public health worldwide and demanded research of new therapeutic approaches (Bonomo et al. 2018). focusing on the lipid One interesting strategy was a biosynthetic pathway—especially the LpxC enzyme—which was so crucial for the creation of lipopolysaccharides—Barb et al., 2019. This work predicted against the LpxC enzyme the binding affinities of 9,12-octadecadienoic acid (Z,Z) methyl ester in combination with several antibiotics, therefore evaluating their prospective efficacy as antibiotics.

Molecular docking studies revealed binding affinities of the examined compounds varying from -6.9 kcal/mol to -9.0 kcal/mol, therefore displaying variants in degree of interaction with the LpxC enzyme. The combination of 9,12-octadecadienoic acid (Z, Z) methyl ester with cefepime (FEP) having the maximum binding affinity (-9.0 kcal/mol) suggested strong interaction and probable inhibitory effects on LpxC. Cefepime was a fourth-generation cephalosporin whose enhanced binding in tandem with the predicted chemical may lead to a synergistic effect deserving of future research. With broad-spectrum action against MDR Gram-negative bacteria.

Combining cefazoline (-8.7 kcal/mol), amoxicillin (-8.6 kcal/mol), and ceftazidime (-8.5 kcal/mol) also showed that the rather strong binding affinities were displayed. These results suggested that beta-lactam antibiotics—especially cephalosporins—may target LpxC in concert with 9,12-octadecadienoic acid (Z,Z) methyl ester, thereby improving the bacterial inhibition (Tommasi et al., 2015). Especially, clindamycin showed a reduced binding affinity (-6.9 kcal/mol), implying less interaction with LpxC, which fit its limited efficacy against Gram-negative bacteria because of the inherent resistance mechanisms (Lewis, 2020).

The expected molecules produced from 9,12-Octadecadienoic acid (Z, Z) methyl ester also showed promise in the combination with trimethoprim/sulfamethoxazole, an antibiotic known for its efficacy against several MDR Gram-negative infections. Though the binding affinity of this combination was not indicated precisely, its predicted interaction hints to a likely role in bacterial inhibition requiring more experimental support.

These findings suggested that 9,12-octadecadienoic acid (Z, Z) methyl ester may increase the efficiency of many antibiotics against multi-drug resistance Gram-negative bacteria s by interacting with the LpxC enzyme. More in vitro and *in vivo* research was needed to verify these computational predictions and evaluate the pharmacokinetics, toxicity, and probable clinical applications of these medicine combinations (Bonomo et al., 2018).

Conclusion

We may conclude from the above findings that the *Klebsiella pneumoniae* strain KP2211 was resistance to most common antibiotics as Amoxycillin, Cefepime, Ceftazidime, and Clindamycin on the other hand, polyhydroxyalkanoates(PHAs) reported as drug delivery system so, Research on the predicted compound from in silico synergistic combination between antibiotic and the PHAs compound has yielded positive indicators for promising way in drug discovery against pneumoniae disease and decrease multi drug resistance gram negative bacteria

Ethical approval

The study was approved by Arish University, Egypt (REC number: 00000114). This was done following the ethical standards of the 1964 Helsinki Declaration and its later comparable ethical standards or amendments, as well as the ethical standards of the national and/or the institutional research committee. The maneuver was explained, and written consent was taken from all couples before starting the study. This work included 1 human sputum sample from Arish General Hospital (AGH).

Conflict of interest

The authors declare that they have no conflict of interest.

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