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Staphyloxanthin and Meropenem Effects on Some Capsular Genes Expressions of *Klebsiella pneumoniae* Isolated from UTIs.

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Abstract

Klebsiella pneumoniae has become one of the most common etiologic agents in the context of recurrent UTI. Capsule is the earliest recognized factor determining bacterial pathogenicity. This study aimed to test the effects of staphyloxanthin, meropenem and a combination of them on capsule genes of *Klebsiella pneumoniae.* Eighty-eight gram negative bacterial isolates were collected from 150 urine specimens between September and November of 2022.after cultivation on MacConkey agar medium. Oxidase (negative) and catalase (positive) tests were performed. In addition, all isolates were found to be non-motile when their motility was further examined. The results presented that 77 (51.3%) could ferment lactose (lactose fermenter), while 30 isolates (20%) of them were confirmed as *K. pneumonia.* The antibiotics sensitivity test of (30) *K. pneumoniae* revealed that 28 (93%) of isolates were sensitive to meropenem, and about 20 (67%), 16 (53%), 8 (27%), 7 (23%), 5 (17%) and 4 (13%) were sensitive toward amikacin, ciprofloxacin, trimethoprim, cefepime, tetracycline and Augmentin respectively. *S. aureus* (producers of staphyloxanthin) isolates were taken; the staphyloxanthin pigment was extracted, purified by Millipore filter (0.22 mm). Conventional PCR results revealed the presence of *galF* gene (141 bp) in all eight tested isolates (100%). In addition, the *wzi* gene (177bp) was found in seven isolates (87.5%). According to the quantitative real-time PCR results, most treatments resulted in downregulation of *galF* and *wzi* genes expression, whereas some treatments resulted in upregulation.

Keywords: Meropenem, Staphyloxanthin, *galF*, *wzi*, *Klebsiella pneumonia*.

تأثير صبغة الستافلوزانثين و الميروبينم على تعبير بعض جينات الكبسولة في بكتريا الكليبسيلا الرئوية المعزولة من مرضى التهاب المسالك البولية

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الخلاصة

أصبحت بكتريا الكليبسيلا الرئوية أحد أكثر العوامل شيوعًا و المسببة لالتهاب المسالك البولية المتكرر. الكبسولة هي العامل الاسبق المعروف والمحددلإمراضية البكتيرية. هدفت هذه الدراسة إلى اختبار تأثير صبغة الستافلوزانثين و الميروبنيم ومزيجهما على جينات كبسولة الكليبسيلا الرئوية. تم الحصول على 88 عزلة بكتيرية سالبة الجرام من 150 عينة بول بين ايلول وتشرين الثاني 2022، بعد الزراعة على وسط أجار MacConkeyتم إجراء اختباري الأوكسيديز والكتلايز. بالإضافة إلى ذلك ، وجد أن جميع العزلات غير

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متحركة عند فحص الحركة بشكل أكبر .وأظهرت النتائج أن 77 (51.3٪) يمكن أن تخمر اللاكتوز (تخمير اللاكتوز) ، بينما تم تأكيد 30 عزلة (20٪) منها على أنها الكليبسيلا الرئوية. أظهر اختبار حساسية المضادات الحيوية لـ (30) من الكليبسيلاالرئوية أن (93%)28 من العزلات كانت حساسة لعقار الميروبينم، 20(67%)، 16(53%)، 8(27%)، 7(23%)، 5(17%) و 4(13%) كانت حساسة تجاه السيبروفلوكساسين، أميكايسين، سيفبيم، تتراسايكلين، أكمنتين وتريميثوبريم،على التوالي. تم أخذ عزلات المكورات العنقودية الذهبية (منتجة لصبغة الستافلوزانثين) ، وتم استخلاص صبغة الستافلوزانثين وتنقيتهابواسطة مرشِح الثقوب الدقيقة (0.22 ملم). أظهرت نتائج تفاعل البوليمراز المتسلسل التقليدية وجود جين *galF*(141pb) في جميع العزلات الثماني المختبرة (100٪). بالإضافة إلى ذلك ، وجد جين *wzi* (177bp) في سبع عزلات (87.5٪). وفقًا لنتائج تفاعل البوليمراز المتسلسل الكمية في الوقت الفعلي ، أدت معظم المعالجات إلى تقليل تنظيم التعبير الجيني لـ *galF* و *wzi* بينما أدت بعض العلاجات إلى زيادة التنظيم.

1. Introduction

Through 1883, Friedlander a German pathologist isolated a capsulated *Bacillus* from the lungs of patient who died of pneumonia. *Klebsiella* is called Friedlander's *Bacillus* [1]. Within the genus *Klebsiella, Klebsiella pneumonia* the most important human pathogen, is responsible for numerous infections (most common in nosocomial infections, including UTIs) [2]. The normal flora of the mouth, intestines and skin include this encapsulated gram-negative bacteria [3], Additionally, it is the third most common microorganism to be isolated from blood cultures of sepsis patients [4].

Urinary tract infection, soft tissue infections, endophthalmitis, meningitis and pneumonia are among the illnesses brought on by *K. pneumoniae* [5]. The opportunistic bacteria *K. pneumoniae* is frequently linked to catheter-associated UTIs in hospitalized patients, as well as in community-acquired UTIs in impaired individuals. UTI is the most serious hospital acquired infection [6]. *K. pneumoniae* is one of the "ESKAPE" diseases causative agents and the most prevalent multiple drug resistance (MDR) pathogens worldwide that include six bacterial pathogens [7]. Over many years, *K. pneumoniae* UTIs have generally increased in frequency, importance and morbidity. The development of these bacteria as infections of the urinary tract and as a cause of antibiotic resistance presents a difficult management and therapeutic challenge for the clinicians [8].

K. pneumoniae strains have resistance to numerous lactams, including third- and fourth-generation cephalosporin, penicillin, monobactam and carbapenems, as well as to other classes of antibiotics such fluoroquinolones, aminoglycosides and sulphonamides [9]. Anacidic capsule polysaccharide that *K. pneumoniae* generates, is crucial for the organism survival in the host [10]. A cps locus is made up of several genes that are necessary for (capsular polysaccharide) CPS biosynthesis. At the 5′ end of the cps locus, conserved genes *galF, orf2, wzi, wza, wzb,* and *wzc* encoded proteins that control the translocation and assembly of CPS onto the bacterial surface [11]. The capsule has attracted a lot of interest as it makes bacteria more resistant to medication therapy and hides surface antigens which lessens the immune system's reaction to infection [12].

New methods to limit and restrict bacterial development must be developed as MDR strains of these bacteria have become a significant issue in the treatment of *Klebsiella* infections [13]. *S. aureus* produce Staphyloxanthin (STX)as a secondary metabolite. Staphyloxanthin carotenoid plays the most potent role as antioxidant, anticancer and as antimicrobial activity [14]. A previous study showed its antimicrobial effects against drug

resistant pathogens such as *Klebsiella pneumonia and* other bacteria [15]. This study objective was to investigate how staphyloxanthin affects the genes of capsule that are responsible for the virulence and survival of *K. pneumonia*.

2. Materials and Methods

2.1 Collection of Klebsiella pneumonia Isolates

The midstream of urine was taken and centrifugation was performed for each sample (3000 rpm / 2 minutes). After that a cotton swab was taken from the sediment and transferred to the laboratory. Following that MacConkey agar, a selective medium for *Klebsiella* spp., was used to culture the samples. The samples were then subjected to a 24-hour optimum incubation period at 37°C. Microorganisms were sub-cultured on slants and kept at 20°C in nutrient broth medium with 40% glycerol after being isolated on MacConkey agar and maintained in deep freeze [16].

2.3 Identification of Bacteria

Culture media, including MacConkey agar (MCA) and nutrient agar, were inoculated with the isolates and incubated at 37°C for 18-24 hr. Both morphologically and biochemically suspicious colonies were identified. The initial biochemical tests, which showed the presence of *K. pneumonia* in the bacterial isolates, were followed by using Vitek-2 device to confirm the isolates identification.

2.4 Microscopic Features

Gram staining was used to investigate the suspicious colonies in order to determine the distinctive appearance of bacteria under a light microscope [17].

2.5 Screening for Staphyloxanthin Production by Staphylococcus aureus Isolates

Identification of the highest yielding *S. aureus* isolate and evaluation of its staphyloxanthin production were performed by creating a *S. aureus* isolation inoculum on skimmed milk agar. The culture was kept at 37°C for 72 hr. of incubation. After incubation, the growth appearance with yellowish color indicated a successful outcome.

2.6 Extraction of Staphyloxanthin Pigment

The pigment from *S. aureus* STX was extracted using methanol, with modification [18]. Tryptic soy broth (TSB) was inoculated with a *S. aureus* 24-hour culture and 24 hr. incubation at 37°C with shaking. The supernatants were removed after the bacteria were centrifuged at 10,000 rpm for 10 minutes. The pellets were resuspended since being extracted with methanol for 24 hours at 37°C while being shaken. Following that, the extract was centrifuged for 10 minutes at 10,000 rpm to obtain the supernatants. Staphyloxanthin was purified by sterile Millipore filter, as mentioned by Pelz *et al.* [19]

2.7 Staphyloxanthin Pigment Production and Estimation with UV-Vis Spectroscopy

To estimate the production of staphyloxanthin, a double-beam of UV-Visible spectrophotometer was used to measure the absorbance at 460 nm for the quantitative assessment of the yellow pigment as proposed by Dong, *et al.* [20]. An absorption spectra with a single maximum between 600 and 460 nm best describes the pigments under examination [21].

2.8 Antibiotic Susceptibility Testing

It was done by using the modified Kirby-Bauer approach [22] towards the antibiotics: cefepime, ciprofloxacin, meropenem , amikacin, tetracycline, Augmentin, carbencillin and trimethoprim.

2.9 Determination of Minimum Inhibitory Concentration (MIC) Values

The MIC values of the antibiotics (meropenem) and staphyloxanthin, as well as combination of meropenem with staphyloxanthin, were determined against *K. pneumonia* isolates by using Broth micro-dilution method [23].

2.10 Molecular Assay

2*.10.1 Extraction of DNA*

A commercial DNA extraction kit (G-spin extraction kit) was used to extract the DNA from *K. pneumonia* in accordance with the manufacturer's instructions.

PCR Amplification

OneTaq® 2X Master Mix (NEB® (England)) was distributed thawed at 4°C. The extracted DNA and the primers listed in Table 1, all were vortexed to ensure uniform contents and the PCR mixture was prepared (Table 2). DNA was amplified in a thermocycler PCR apparatus using PCR reaction tubes under the settings shown in Table 30. Gradient PCR was used to optimize the temperature and time of the PCR.

Table 1: Primers sequences.

Gene	Primer	Sequence ´5 →´3	Reference
16srRNA	F	GTAAAGCACTTTCAGCGGGA	This study
	R	CACATCCGACTTGACAGACCG	
wzi	F	TAGCGGAGATTTGGTACTGCC	This study
	R	AGTGGCTCTCTTTTGGTCAGG	
galF	F	AGCTACTGGCGGAAGTGC	[24]
	R	GCAGGACCACCACAAACG	

Table 2: Reaction mixture of PCR.

Component	Volume
OneTaq® 2X Master Mix	12.5 µl
Forward primer	1.5 µl
Reverse primer	1.5 µl
DNA template	5 µl
Nuclease free dH2O	4.5 µl
Final volume	25 µl

Table 3: PCR conditions for capsule genes.

Cycle No.	Stage	Temperature	Time
1	Initial Denaturation	94ºC	3 mins.
38x	Denaturation Annealing Extension	94ºC 55ºC(*galF* gene) 54 ºC(*Wzi* gene) 72ºC	30 sec. 45 sec. 30 sec.
1	Final Extension	72ºC	7 mins.

2.10.2 Electrophoresis

After PCR, agarose gel electrophoresis was used to confirm the results. PCR was utilized to extract all of the DNA characteristics. One gram of agarose was added to a 100 ml beaker of 1X TBE to make agarose gel. DNA ladder and 10 µl of PCR product were loaded into each well in the gel. Gel documentation was employed to collect images and evaluate the bands at the end of the run.

2.10.3 Molecular Detection of 16S rRNA, wzi, galF Genes

This step was carried out as stated in Table 2 with the specific primer as mentioned above in Table 1.

2.10.4 Real-Time – Polymerase Chain Reaction (qRT-PCR)

In a space devoid of nucleases, qRT-PCR should be constructed as stated in Table 4.

Table 4: Reaction mixture of QRT-PCR

Component	Volume
Luna Universal qPCR Master Mix	10 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
Template DNA	5 µl
Nuclease-free Water	3 µl
Final volume	20 µl

2.11 Estimating the Effects of Meropenem sub-MIC and Staphyloxanthin, and Combination of Them on Capsule Genes.

Three isolates were chosen for testing the impact of the meropenem and staphyloxanthin, and combination of meropenem with staphyloxanthin at sub MIC which were determined against *K. pneumonia* isolates on the gene expression of *wzi* and *galF*.

3. Results and Discussion

3.1 Collection and Identification of K. pneumoniae Isolates

Eighty-eight gram negative bacterial isolates were gained from 150 urine specimens after cultivation on MCA medium. Only gram-negative bacterial species can grow on MacConkey agar (MCA) which is a lactose-metabolizing selective and differentiating agar that can further distinct the gram-negative organisms [25]. The results presented that 77 (51.3%) isolates could ferment lactose (lactose fermenter), while 30 isolates (20%) of them which were characterized by large, mucoid, lactose fermenter with pink to red color colonies on MacConkey agar, were suspected *K. pneumoniae*. Oxidase and catalase tests were performed and the findings showed that all bacterial isolates were catalase-positive and oxidase-negative. All isolates were found to be non-motile when their motility was further examined. It was expected that the found 30 bacterial isolates were *K. pneumonia* which was confirmed by Vitek -2 compact. Ballén, *et al.* in their study isolated *K. pneumoniae* in a percentage of 40% [26] and Mustafa, *et al*. isolated *K. pneumoniae* in percentage 30% [27], while it was reported to be about 14.70 % by Lin *et al.* [28].

3.2 Screening of Staphylococcus aureus Isolates for Staphyloxanthin Production

The best staphyloxanthin-producing isolate was chosen among the ten pre-isolates of *S. aureus* to be employed in this study's subsequent experiments. The screening was done in

skimmed milk agar, and for more consistency all isolates were grown under identical conditions for inoculum size, pH, cell count, incubation timeand shaking rate.
The results exhibited that S1 was the best isolate (the color was darker) followed by the S4. While other isolates did not produce pigment. AL-Kazaz *et al.* found that the skimmed milk agar medium had the maximum creation of pigment after 72 hr at 37˚C [29].

3.3 Extraction of Staphyloxanthin Pigment

After extracting dark yellow powder of staphyloxanthin, the sterile Millipore filter was used to purify it partially. After extraction and purification, it is recommended to use the powder immediately or keep it in the fridge in a dark glass plate as the pigment is light sensitive [19].

3.4 Staphyloxanthin Pigment Production and Estimation with UV-Vis Spectroscopy

The outcome indicated that the absorbance of staphyloxanthin pigment under UV was visible at about O.D 460 nm. The results were similar the results obtained by Pelz, A. *et al.* that staphyloxanthin from *S. aureus* [19] had a maximum absorption spectrum at 462 nm.

3.5 The Antibiotic Susceptibility Testing for Klebsiella pneumoniae

A susceptibility test for eight different antibiotics (meropenem, ciprofloxacin, amikacin, cefepime, tetracycline, Augmentin, trimethoprim, carbencillin) was performed on thirty isolates by the disc diffusion method, as recommended by the medical and laboratory standard association guidelines (CLSI, 2023). The results showed that 93%, 67%, 53%, 27%, 23%, 17% and 13% of the isolates were sensitive towards meropenem, amikacin, ciprofloxacin, trimethoprim, cefepime, tetracycline, Augmentin respectively. While 100% of the isolates were resistant to carbencillin (Figure 1).

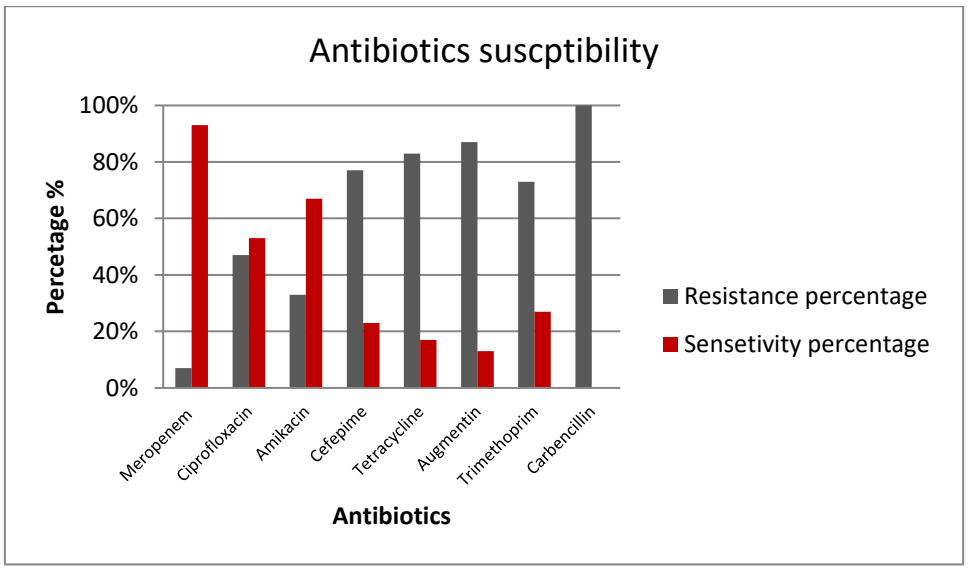


Figure 1: antibiotic susceptibility percentage of *K. pneumoniae* isolates to 8 antibiotics.

3.6 Minimal Inhibitory Concentration Test of Staphyloxanthin, Meropenem, Combination of them Against K. pneumonia

As recommended by the CLSI, 2023; microdilution technique was used to investigate the MIC for staphyloxanthin, meropenem and their combination by using eight *Klebsiella pneumoniae* isolates. (Figure 2).

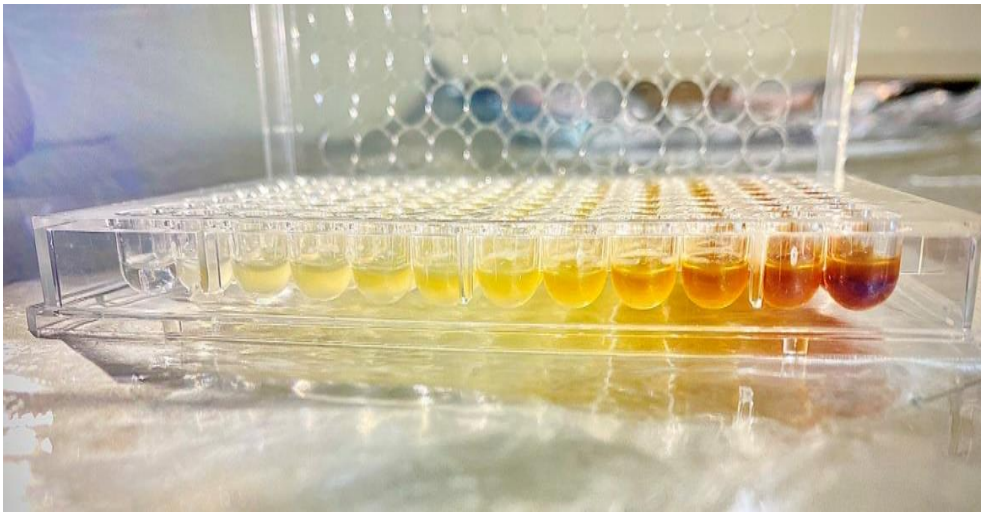


Figure 2: Microdilution plate to determine MIC of Staphyloxanthin and antibiotics

The results presented that the MIC of meropenem was 2µg/ml and that of staphyloxanthin was 250 mg/ml. Whereas MIC of combination between staphyloxanthin and meropenem distributed between 1.9 and 7.8 mg/ml for staphyloxanthin, and between 0.25 and 1 µg/ml for meropenem against eight isolates. It was clear from the results that MIC of staphyloxanthin and meropenem decreased for both against the eight isolates of *K. pneumonia* by the synergistic effect. It decreased from 2 µg/ml to 1-0.25 µg/ml for meropenem and from 250 mg/ml to 1.9-7.8 mg/ml for Staphyloxanthin against the eight isolates (Table 5).

Table 5: MIC of Staphyloxanthin (S), meropenem (M), combination between Staphyloxanthin and meropenem (S/M)) for *K. pneumoniae.*

Isolates	Meropenem	Staphyloxanthin	Combination of S/M
1	2µg/ml	250mg/ml	3.9 mg/ml / 0.5 µg/ml
2	2µg/ml	250mg/ml	1.9 mg/ml / 0.25 µg/ml
3	2µg/ml	250mg/ml	1.9 mg/ml / 0.25 µg/ml
4	2µg/ml	250mg/ml	3.9 mg/ml / 0.5 µg/ml
5	2µg/ml	250mg/ml	3.9 mg/ml / 0.5 µg/ml
6	2µg/ml	250mg/ml	7.8 mg/ml / 1 µg/ml
7	2µg/ml	250mg/ml	7.8 mg/ml / 1 µg/ml
8	2µg/ml	250mg/ml	1.9 mg/ml / 0.25 µg/ml

One of the most often used antibiotic for treating *K. pneumoniae* infections, meropenem is a member of the carbapenem family of drugs and exhibits broad-spectrum *in vitro* resistance to both gram-positive and -negative bacteria [30]. It easily passes through the cell walls of the majority of gram-negative and -positive bacteria to reach the penicillin-binding protein (PBPS) that it is looking for and demonstrates stability against hydrolysis by the majority of lactamases.

Bulik *et al*. found that the BMD MIC test range was 0.06 µg/ml to 64 µg/ml [31], while Cojutti *et al*. stated that the a meropenem (MIC≤2 µg /L) against *K. pneumonia*[32]. Hence, we noted that such substances (pigments of bacteria) have the potential to improve the effects of antibiotics and provide a choice of alternative to antibiotics. Numerous studies comparable to this one have demonstrated the use of pigments such as staphyloxanthin, prodigiosin and pyocyanin against a wide range of microorganisms [15, 29, 33, 34].

3.7 Molecular Analysis

3.7.1 DNA Extraction

DNA of eight isolates of *K. pneumoniae* was successfully extracted from overnight cultures of isolates. Gel electrophoresis was used to establish that the DNA bands were intact.

3.7.2 Detection of 16srRNA Gene

Genus-then-species identification for isolates is one of the most intriguing potential uses of the 16S rRNA gene sequence in informatics [35]. In terms of bacterial taxonomy, 16S rRNA gene sequencing is quite significant [36]. The results indicated presence of this gene (Figure3)

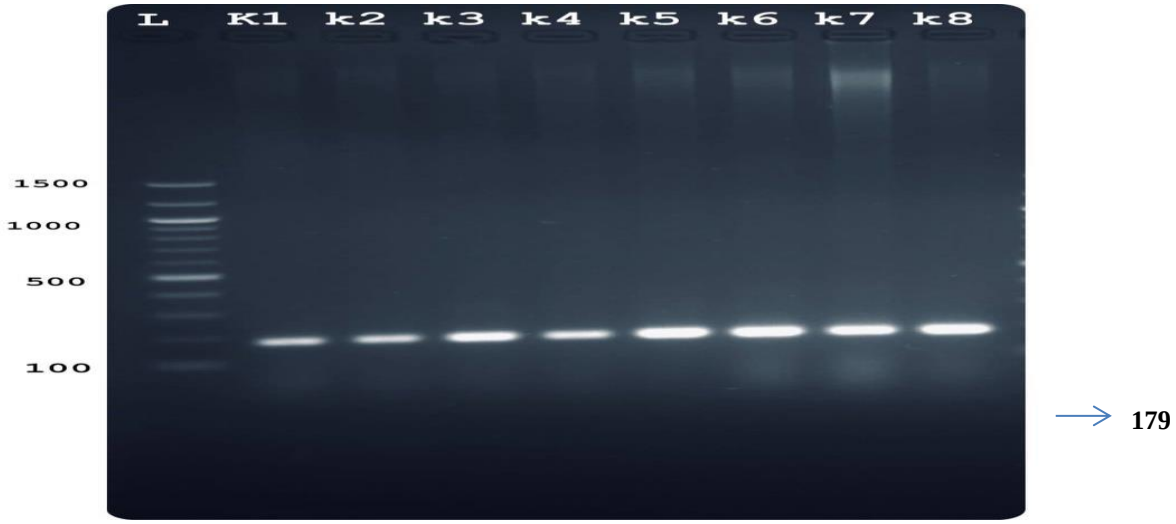


Figure 3: The 16srRNA gene of *K. pneumoniae* was electrophoresed on an agarose gel with an amplicon of (179 bp). 2% agarose gel and TAE 1X were used for the electrophoresis which consumed 90 minutes (RedSafe dye was added as a DNA staining dye. L: Ladder marker, K: Isolates no.).

3.7.3 Detection of galF Gene

The constitutional genes *galF* were amplified by PCR for eight isolates using the *galF* primer, and bands were verified by gel electrophoresis. The findings showed that eight *K. pneumoniae* isolates had the *galF* gene [24] (Figure 4).

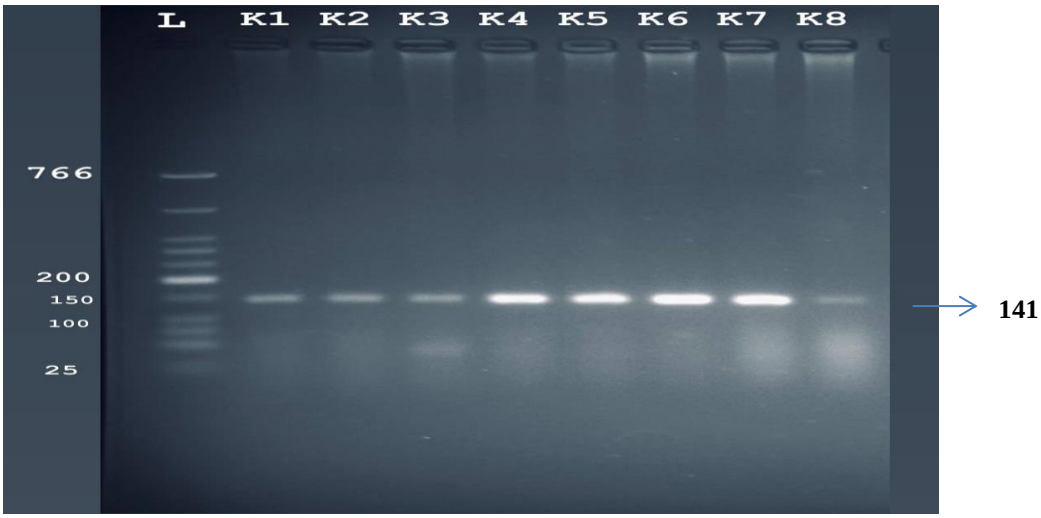


Figure 4: The *galF* gene of *K. pneumoniae*, which controls capsule regulation, was electrophoresed on an agarose gel with an amplicon of (141bp). 2% agarose gel and TAE 1X were used for the electrophoresis which consumed 90 minutes (RedSafe dye was added as a DNA staining dye. L: Ladder marker, K: Isolates no.).

3.7.4 Detection of wzi Gene

The constitutional genes *wzi* were amplified by PCR for eight isolates using the *wzi* primer, and bands were verified by gel electrophoresis. According to the findings seven *K. pneumoniae* isolates had the *wzi* gene [37] (Figure 5).

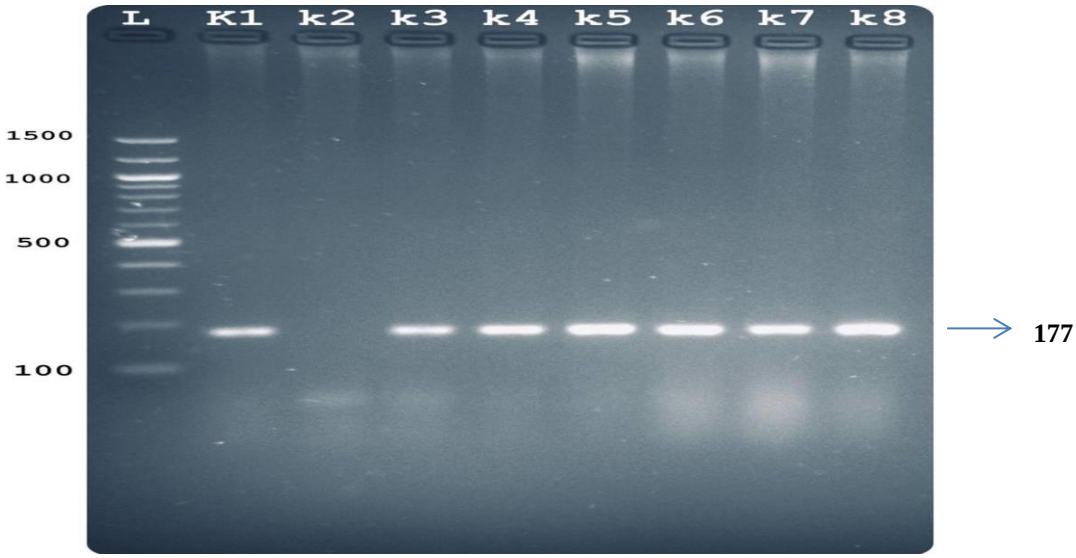


Figure 5: The *wzi* gene of *K. pneumoniae*, which controls capsule regulation, was lectrophoresed on an agarose gel with an amplicon of (177bp).2% agarose gel and TAE 1X were used for the electrophoresis, which consumed 90 minutes (RedSafe dye was added as a DNA staining dye. L: Ladder marker, K: Isolates no.).

3.7.5 Real time-qPCR

RNA was extracted from the chosen isolates of *K. Pneumoniae* (3, 4, 6) and grown in Muller Hintone broth as control. Additionally, these samples received a sub-MIC of staphyloxanthin (125 mg/ml) and sub-MIC of meropenem (1 µg/ml), and synergistic between staphyloxanthin and meropenem sub-MIC (0.9 ,1,9 and 3.9 mg/ml for staphyloxanthin, 0.125 ,0.25 and 0.5 µg/ml for meropenem, for three isolates of *K. pneumonia* as mentioned in Table 6. Total RNA was extracted from the samples using the Qubit™ RNA HS Assay Kit.

Table 6: Sub-MIC of meropenem and Staphyloxanthin, also combination of them for three isolates of *K. pneumoniae.*

Isolates	Meropenem	Staphyloxanthin	Combination of S/M
3	1µg/ml	125mg/ml	0.95 mg/ml/0.125 µg/ml
4	1µg/ml	125mg/ml	1.9 mg/ml/0.25 µg/ml
6	1µg/ml	125mg/ml	3.9 mg/ml/0.5 µg/ml

3.8 Estimating the Effects of sub-MIC of Meropenem and Staphyloxanthin, also Combination of Them on Capsule Genes

Treating the three isolates (k3, k4 and k6) with sub-MICs of staphyloxanthin, meropenem, and combinations of them (Table 6), the results exhibited that the staphyloxanthin treatment showed significant decrease in the gene expression levels for the *galF* gene, 0.03, 0.07, and 0.16 fold of the three isolates respectively. While another gene, the *wzi* gene, showed 0.02, 0.0001, and 0.0006 folds of the three isolates respectively Table (7, 8).

With meropenem treatment the result showed increase in the gene expression levels of about 1.59 fold for first the isolate and a slight decrease in the gene expression levels of about 0.4

and 0.5 folds in the other two isolates of the *galF* gene, while demonstrating increase of about 1.31 in the gene expression levels in the first isolate and slightly decreasing in the gene expression levels (about 0.16 and 0.05) in the other two isolates respectively of the *wzi* gene (Table 7, 8).

Currently carbapenems are suggested for the treatment of infections linked to UTIs caused by ESBL-PE [38, 39]. Meropenem acts by quickly entering bacterial cells and interfering with the production of essential components for the cell wall resulting in cell death [40]. The threat of carbapenem-resistant Enterobacterales (CRE), which are rapidly spreading around the world, is a serious threat to the public health [41]. Thus, the clinical use of carbapenems has been restricted by the rise in antimicrobial resistance [42]. It is therefore crucial to offer alternative antibiotic for the treating MDR infections.

The effects of antibiotic exposure varied between the isolates. This fluctuating impact of meropenem on genes suggests that its impact is strain-specific. These findings correspond with those of various other studies. Meropenem has varying impacts on various strains and genes, according to prior studies that utilized it to treat bacteria and found variable results against diverse strains. It had both up and a down-regulation actions [43, 44].

The combination of them exhibited down-regulation in all isolates and of both genes, such as down-regulation of about 0.05, 0.2 and 0.3 folds in the three isolates respectively of the *galF* gene and about 0.03 fold in the first isolate, and 0.002 in the last two isolates of the *wzi* gene (Table 7, 8).

Despite the combination of staphyloxanthin and meropenem revealing a synergistic effect by the minimal inhibitory concentration tests in which the MIC of both greatly decreased, we noted that genomically they were not same. The results exposed a moderate difference between staphyloxanthin and meropenem. However, in this study they still have a great impact on genes. Similar to comparable strategies, synergistic effects on genes have shown decrease in the relative gene expression levels of genes, especially bacterial pigment with antibiotics [33, 45].

Table 7: Fold change of *galF* gene and *16srRNA* gene in *K. pneumoniae* at sub-MICs for (Staphyloxanthin (S), Meropenem (M) and synergism between of them (S/M))

Isolate (1)	**Ct of *16srRNA***	**Ct of *galF***	**ΔCt**	**ΔΔCt**	**folding**
Control	10.91	17	6.09	0.0	
S	13.66	24.96	11.3	5.2	0.03
M	12.05	17.75	5.7	-0.39	1.59
S/M	9.66	20.52	10.8	4.7	0.05
Isolate (2)	**Ct of *16srRNA***	**Ct of *galF***	**ΔCt**	**ΔΔCt**	**folding**
Control	15.93	31.73	15.8	0.0	
S	14.35	34.01	19.66	3.86	0.07
M	13.57	30.71	17.14	1.3	0.4
S/M	13.81	31.67	17.86	2.06	0.2
Isolate (3)	**Ct of *16srRNA***	**Ct of *galF***	**ΔCt**	**ΔΔCt**	**folding**
Control	15.93	31.73	15.8	0.0	
S	13.41	31.85	18.44	2.6	0.16
M	16.28	31.96	15	0.8	0.5
S/M	13.96	31.16	17.2	1.4	0.3

Table 8: Fold change of *wzi* gene and *16srRNA* gene in *K. pneumoniae*at sub-MICs for (Staphyloxanthin(S), meropenem(M) and synergism between of them(S/M))

Isolate (1)	**Ct of *16srRNA***	**Ct of *wzi***	**ΔCt**	**ΔΔCt**	**folding**
Control	10.91	17.42	6.51	0.0	
S	13.66	25.08	11.42	4.91	0.02
M	12.05	17.89	5.84	-0.67	1.31
S/M	9.66	20.47	10.81	4.3	0.03
Isolate (2)	***16srRNA***	**Ct of *wzi***	**ΔCt**	**ΔΔCt**	**folding**
Control	15.93	19.36	3.43	0.0	
S	13.57	29.79	16.22	12.7	0.0001
M	13.81	19.85	6.04	2.61	0.16
S/M	14.35	26.29	11.94	8.51	0.002
Isolate (3)	**Ct of *16srRNA***	**Ct of*wzi***	**ΔCt**	**ΔΔCt**	**folding**
Control	15.93	19.63	3.43	0.0	
S	13.96	27.98	14.02	10.5	0.0006
M	13.41	21.15	7.74	4.31	0.05
S/M	16.28	28.26	11.98	8.55	0.002

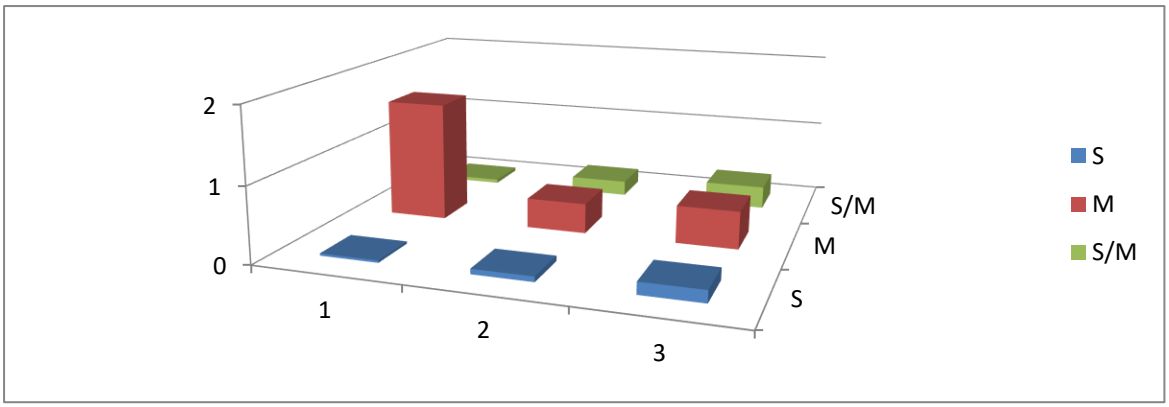


Figure 6: Fold change of *galF* gene (Staphyloxanthin (S), Meropenem (M) and synergism between of them (S/M))

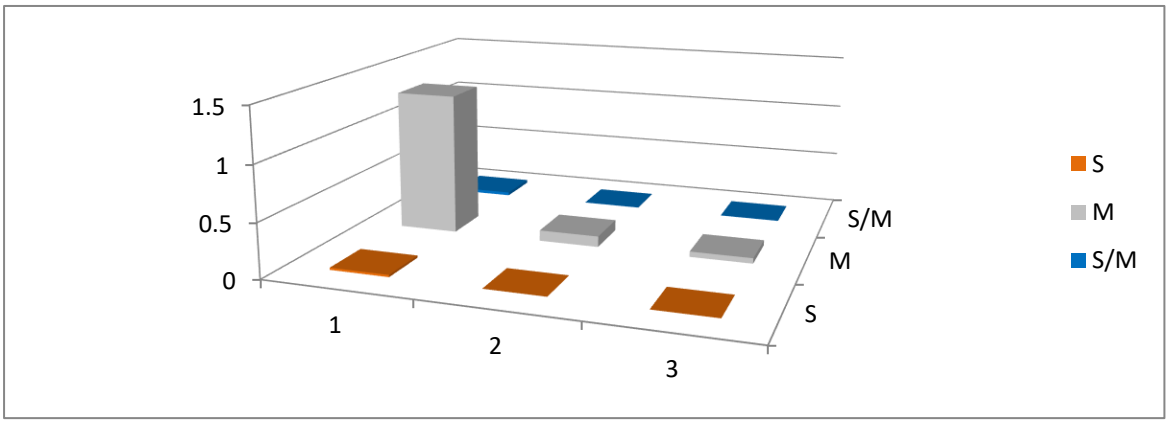
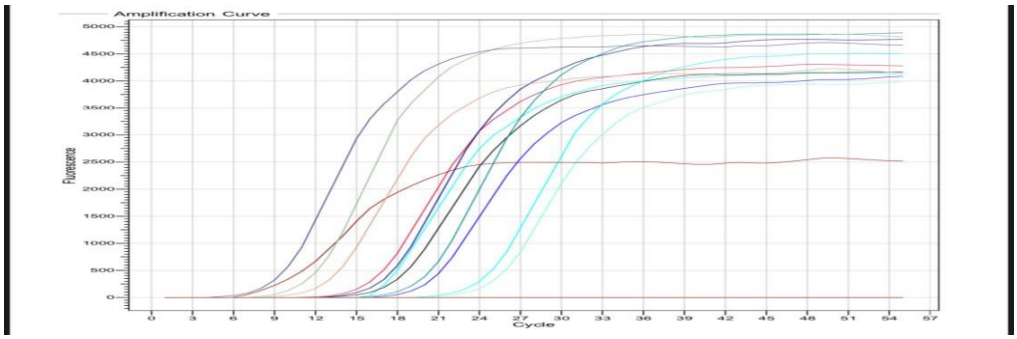


Figure (7): Fold change of *Wzi* gene, (Staphyloxanthin(S), Meropenem (M) and synergism between of them(S/M))



Sample Id	Color
wzi-1	
wzi-2	
wzi-3	
wzi-4	
wzi-5	
wzi-6	
wzi-7	
wzi-8	
galF-1	
galF-2	
galF-3	
galF-4	
galF-5	
galF-6	
galF-7	
galF-8	
16S rRNA-1	
16S rRNA-2	
16S rRNA-3	
16S rRNA-4	
16S rRNA-5	
16S rRNA-6	
16S rRNA-7	
16S rRNA-8	

Figure 8: Melt curve following treatment for isolates 4, 6 for 16srRNA, *wzi*, and *galF* genes.

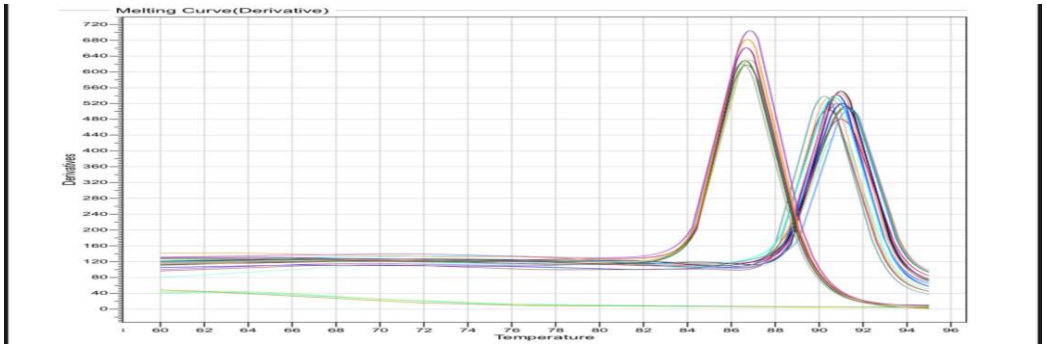
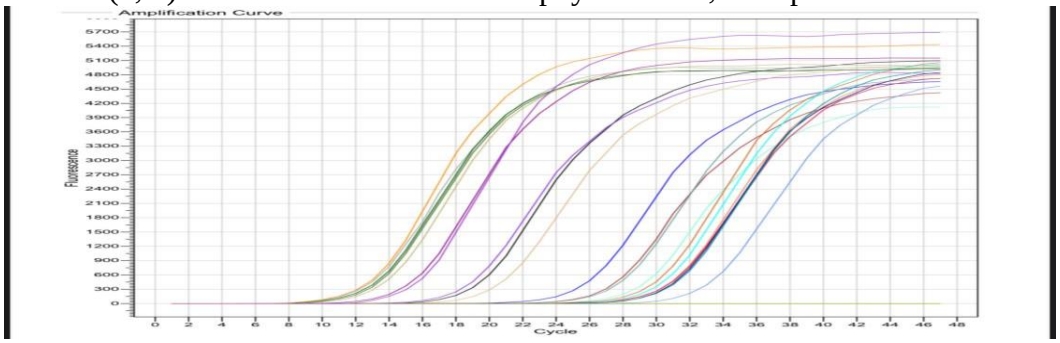


Figure 9: *galF*, *wzi*, and 16S rRNA gene amplification curves in *K. pneumoniae* isolates (4, 6) treated with sub-MIC of staphyloxanthin, meropenem and their combination.



Sample Id	Color
wzi-1	
wzi-2	
wzi-3	
wzi-4	
galF-1	
galF-2	
galF-3	
galF-4	
16S rRNA-1	
16S rRNA-2	
16S rRNA-3	
16S rRNA-4	

Figure 10: *galF*, *wzi* and 16S rRNA gene amplification curves in *K. pneumoniae* isolate (3) treated with sub-MIC of taphyloxanthin, meropenem and their combination.

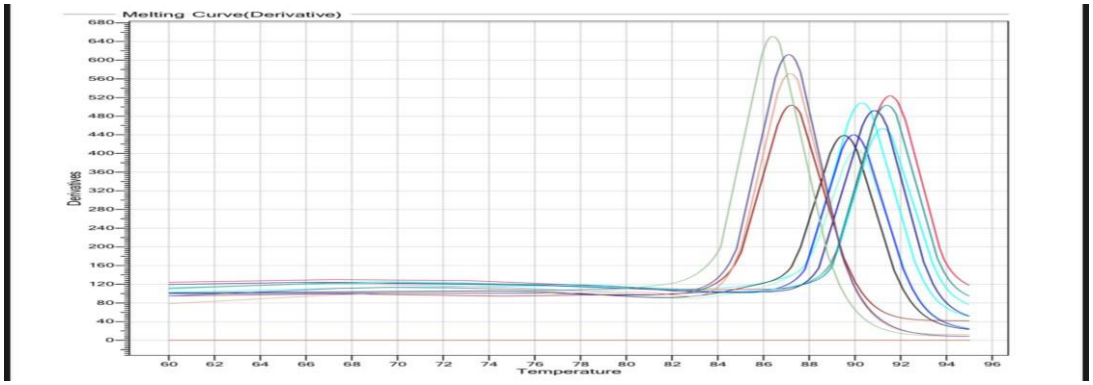


Figure 11: Melt curve following treatment for isolate (3) for 16srRNA, *wzi* and *galF* genes.

Although both Staphyloxanthin and Meropenem have downregulated genes expression , but it's better to use staphyloxanthin as alternative to Meropenem or in combination with it to avoid bacterial resistance toward the antibiotic which is considered one of the first line treatment for UTIs.

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