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التحري عن بعض جينات الضراوة لبكتريا *Klebsiella pneumoniae* المعزولة من عينات سريرية ودراسة التأثير التآزري للمعزز الحيوي ومضاد السبروفلوكساسين على التعبير الجيني

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Detection of some virulence genes in *Klebsiella pneumoniae* isolated from clinical samples and studying the synergistic effect of probiotic and Ciprofloxacin on gene expression

A Thesis

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1-المقدمة Introduction

تعد بكتيريا الكليبسيلا الرئوية *Klebsiella pneumoniae* أحد أفراد العائلة المعوية Enterobacteriaceae وهي من الممرضات الانتهازية Opportunistic pathogens المتوطنة، وتنتقل نتيجة لاستغلالها ضعف مناعة الجسم خلال المرض لإحداث الإصابة (Chen وآخرون، 2020).

تنتشر بكتيريا *K. pneumoniae* على نطاق واسع في البيئة، وتسبب مشاكل اقتصادية وصحية في الإنسان، وتعزل من مواطن مختلفة مثل أجزاء مختلفه من جسم الإنسان والحيوانات، ومياه المجاري، والبحيرات، والمياه العذبة والمالحة (Choby وآخرون، 2020).

توجد هذه البكتيريا كنبيت طبيعي Normal flora في الفم والجلد والأمعاء للإنسان ولها القدرة على إحداث التهابات بكتيرية شديدة، فهي تعد المسبب الثاني لعدوى المجاري البولية Urinary tract infection والالتهابات الرئوية Pneumonia المرتبطة بالمستشفيات والرعاية الصحية والإسهال Diarrhea والحروق Burns والجروح Wounds، كما يمكنها أن تدخل إلى مجرى الدم Blood stream، وتسبب تجرثم الدم Bacteremia، وتسبب أيضاً التهاب السحايا Meningitis وخراج الكبد القيحي Purulent liver abscess بعد بكتيريا إشريشيا القولون *Escherichia coli* (González، 2021 ؛ Duraye، 2022) .

تمتلك بكتيريا *K.pneumoniae* العديد من عوامل الضراوة التي تمثل المقياس لقدرة البكتيريا على غزو الجسم وإحداث الإصابة في جسم المضيف وتُسهم في جعل البكتيريا أكثر شراسة وقدرة على تجاوز الخطوط الدفاعية الأساسية في الجسم وكذلك زيادة مقاومتها للمضادات الحيوية المصنعة (محمد، 2022).

تعد المحفظة Capsule أحد عوامل الضراوة المهمة في بكتيريا *K.pneumoniae* فهي المسؤولة عن إظهار المستعمرات بشكل لامع وبقوام مخاطي mucoid على سطح الأكار، وتساعد على تثبيط عملية البلعمة phagocytosis عندما تكون البكتيريا في حالة تماس مع الخلايا البلعمية، وتمنع إرتباط المتمم مع الغشاء الخلوي للبكتيريا (Levinson، 2016).

توجد العديد من الجينات المسؤولة عن تكون المحفظة في بكتيريا *K.pneumoniae* والتي منها *K1* ، *K2* ، *mag*A ، *rmp*A ، ويعد جين *rmp*A في هذه البكتيريا المسؤول عن فرط اللزوجة المخاطية، وإن البكتيريا التابعة للنمطين *K1* و*K2* مسؤولة عن معظم الأمراض المكتسبة في المستشفيات كالإصابات التنفسية (Al-Hasnawi، 2020)، إن من المسببات الأساسية لانتشار هذه البكتيريا في القناة المعوية هو نتيجة لاستقرارها في القناة الهضمية للمرضى الراقدين في المستشفى ووحدات العناية المركزة وكذلك إنتشارها عن طريق أيدي العاملين بالعناية الصحية والأجهزة الطبية، بالإضافة إلى الاستخدام المتكرر للأجهزة والأدوات التي تكون على تماس مباشر مع المريض (Predic وآخرون، 2020).

تمتلك بكتيريا *K.pneumoniae*، مضخات دفق Efflux pumps وهي من آليات المقاومة في هذه البكتيريا، وهي ناقلات بروتينية توجد في غشاء الخلية، والتي تؤدي دوراً مهماً في نقل وإخراج المواد المختلفة لذلك فهي وسيلة مهمة للمقاومة البكتيرية للمضادات الحيوية، إذ تعمل هذه الناقلات على نقل العديد من المواد خارج الخلية، مثل المعقمات والمضادات وغيرها، وهناك عدة جينات مسؤولة عن تكون مضخات الدفق في بكتيريا *K.pneumoniae* مثل (*acr*A, *acr*B, *oqx*A, *oqx*B, *qnr*A, *qnr*B, *qnr*S) وغيرها (السعدي، 2019).

Summary

Two hundred fifty clinical samples were collected from patients suffering from different diseases, and these samples included urine (121) samples, sputum (78), wounds (34), and burns (17). The sample was collected from December 2022 to June 2023 from the Baquba Teaching Hospital / Diyala. All samples were subjected to bacterial culture, and the results showed that (158) samples gave positive results while (92) samples gave negative results; the isolates were identified by microbiological methods and conformed to the VITEK2 compact system.

The results showed that 57 (36.08%) isolates belonged to *Klebsiella pneumonia*e, as it was in urine 35 (28.92%), sputum 15 (19.23%), wounds 5 (14.70%) and burns 2 (11.76%).

The results of the antimicrobial susceptibility test showed that *K. pneumoniae* isolates were resistant to β-lactam antibiotics (Ampicillin 100%, piperacillin 100%, Cefoxitin87.71%, Imipenem26.31, Meropenem 28.07%), the resistant for Aminoglycosides (Gentamicin 40.35%, Amikacin 50.87%, Streptomycin 80.70%), for Quinolones (Levofloxacin 35.08%, Ofloxacin 31.57%) while for Trimethoprime-sulfathouxazole the resistance has reached (64.91%). The results of the antimicrobial susceptibility test showed that 20 (35.08%) isolates of *K. pneumoniae* were multidrug-resistant (MDR), and 4 (7.01%) isolates were resistant to three antibiotic groups (XDR).

The minimum inhibitory concentration (MIC) of the antibiotic and the probiotic was determined, as the value (MIC) of the antibiotic Ciprofloxacin ranged from (8-1024) micrograms/ml, and when the isolates were treated synergistically with the antibiotic and the probiotic, they were mixed in a 1:1 ratio.

The results of the molecular detection of the *16SrRNA* gene showed that all 22 *Klebsiella pneumonia* isolates (100%) possessed this gene.

Molecular detection using polymerase chain reaction (PCR) technology showed two capsular genes for (22) *K. pneumoniae* isolates under study. It was found that only one isolate possessed the *K1* gene at a rate of (4.54%), while the results of the current study showed that all *K. pneumoniae* isolates did not possess the *rmp*A gene at a rate of (0%).

The results of molecular detection of a group of efflux pump genes (*acr*A, *oqx*A, *oqx*B) in this bacteria showed that all *K. pneumoniae* (22) multiple antibiotic-resistant (MDR) isolates possessed all of these genes (100%).

As for antibiotic genes, the results of molecular detection of these genes (*gyr*A, *qnr*B) showed that all (22) multi-resistant (MDR) isolates of *K. pneumoniae* bacteria possess the *gyr*A gene at a rate of 100%. One (6) of the multi-resistant (MDR) isolates of *K. pneumoniae* bacteria possess the *qnr*B gene at a rate of 22.72%.

Lactobacillus spp. was diagnosed. Microscopically and by biochemical tests, this bacterium was used as a probiotic after treating it with two isolates of *K. pneumoniae* bacteria.

Gene expression was measured by using quantitative real-time polymerase chain reaction (RT-PCR) for the genes (*gyr*A (*Oqx*B, *Acr*A, *K*1) for selected multi-resistant *K. pneumoniae* isolates from different sources, and these isolates were (U4) Isolated from Urine and (S2) isolated from sputum, and after calculating the value of Folding = $2^{-\Delta\Delta CT}$, the results showed that all of these genes showed gene expression, as the average value of Folding was (1). To determine the effect of probiotics on the gene expression of the gene

*gyr*A for the two isolates, and after treating them with a series of dilutions of the probiotic, the minimum sub-inhibitory concentration (subMIC) value was calculated. The current study showed an inhibition in the gene expression of the *gyr*A gene for the two isolates (U4, S2) after treatment with probiotics and compared to Control. Gene expression results for the identical two isolates after being treated with probiotics, ciprofloxacin, and a synergistic of probiotics and ciprofloxacin, and compared to the two isolates before treatment, as there was an inhibition in gene expression for the isolate (U4). After treating it with probiotics, ciprofloxacin, and a synergistic of probiotics and ciprofloxacin, there was an increase in gene expression for the isolate (S2) after treating it with ciprofloxacin, and an inhibition in gene expression after treating this isolate with probiotics, a synergistic of probiotics, and ciprofloxacin. The average value of Folding = $^{2\wedge(-\Delta\Delta CT)}$ for the two isolates before treatment (1) and after treating the two isolates (U4, S2) with probiotics was 0.79 and 0.17, after treating the identical two isolates with ciprofloxacin 1.40 and 0.13, and after treating them with a synergistic of probiotics and ciprofloxacin 0.49 and 0.24.

The results of the current study showed in the gene expression of the *K*1 gene for the isolate (U4) after treating it with the same treatment, as there was an increase in gene expression after treating it with probiotics, ciprofloxacin, and a synergistic of probiotics and ciprofloxacin compared to the isolate before treatment, as the average value was Folding $^{= 2\wedge(-\Delta\Delta CT)}$ For the two isolates before treatment (1) and after treatment of the isolate (U4) with probiotics, 2.71, after treatment with ciprofloxacin, 12.52, and after treatment with a synergistic of probiotics and ciprofloxacin, 7.04.

As for the *Acr*A gene, there was an inhibition in gene expression for the two isolates (U4, S2) after treatment with probiotics and compared to Control. Gene expression results for the identical two isolates after treatment with probiotics, ciprofloxacin, and a synergistic of probiotics and ciprofloxacin, and compared to the two isolates before treatment, as there was an inhibition in gene expression for the isolate (U4). After treating it with probiotics, ciprofloxacin, and a synergistic of probiotics and ciprofloxacin, there was an increase in gene expression for the isolate (S2) after treating it with ciprofloxacin, and an inhibition in gene expression after treating the isolate with probiotics, and synergistic of probiotics, and ciprofloxacin, as the average value was Folding $^{= 2\wedge(- \Delta\Delta CT)}$ for the two isolates before treatment (1) After treating the two isolates (U4, S2) with probiotics 0.02 and 0.08, after treating the identical two isolates with ciprofloxacin 1.84 and 0.08, and after treating them with a synergistic of probiotics and ciprofloxacin 0.55 and 0.23.

The current study showed an inhibition in the gene expression of the *Oqx*B gene for the two isolates (U4, S2) after treatment with probiotics and compared to Control. Gene expression results for the identical two isolates after treatment with probiotics, ciprofloxacin, and a synergistic of probiotics and ciprofloxacin, and compared to the two isolates before treatment, as there was an inhibition in the gene expression of the two isolates (U4, S2).) after treating them with probiotics, ciprofloxacin, and synergistic of probiotics and ciprofloxacin, as the average value was Folding = 2^(-ΔΔCT) for the two isolates before treatment (1), and the inhibition for the two isolates (U4, S2) after treatment with probiotics was 0.51 and 0.25. The inhibition for the two isolates after treatment with ciprofloxacin was 0.89.

And 0.15, and after treating them with a synergistic of probiotics and ciprofloxacin, 0.39 and 0.71.

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