جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية التربية للعلوم الصرفة

الكشف الجزيئي لبعض عوامل الضراوة وتاثير المعزز الحيوي لبكتريا Lactobacillus sp ضد بكتريا *Klebsiella pneumoniae* متعددة المقاومة للمضادات الحيوية المعزولة من اصابات المسالك البولية

اطروحة مقدمة الى

مجلس كلية التربية للعلوم الصرفة

جامعة ديالى كجزءًا من متطلبات نيل درجة دكتوراه فلسفة

في علوم الحياة/ علوم الحياة

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بكالوريوس علوم حياة / كلية العلوم / الجامعة المستنصرية 2001

ماجستير احياء مجهرية / كلية التربية للعلوم الصرفة / جامعة ديالى 2006

بإشراف

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1443هـ **2021م**

Introduction

Klebsiella pneumoniae is Gram-negative bacilli, lactose fermenter, non motile, aerobic and Facultative anaerobic, capsulated (Jawetz *et al*., 2019). Found normally in digestive system of human and can cause opportunistic infection and Nosocomial infection (Hossein zadeh *et al*., 2018) such as, Pneumonia, Urinary Tract Infections(UTIs), Bacterimia, Purulent liver abscess, diarrhea, Wounds and Burns (Bhuiyan, 2016).

K. pneumoniae is a clinically relevant pathogen that has the propensity to acquire Multi-Drug Resistance (MDR). Thus, limiting the therapeutic options for treating related infections such as (UTIs), pneumonia, meningitis, and blood stream infections (Gonçalves *et al*., 2017). Urinary tract infection is one of the most commonly diagnosed infections in both genders and across all age groups in the world (Garza-Ramos *et al*., 2018).

K. pneumoniae is classified among the most important microorganisms contributing UTI as a Gram-negative bacterium, responsible for hospital-acquired infections (Flores *et al*., 2016). Due to the excessive and improper use of antibiotics and the spread of organisms that produce extended-spectrum β-lactamases (ESBLs), the emergence of multidrug-resistant uropathogens *K. pneumoniae*, is consider the second most frequent etiological agent involved in community-acquired infections (Dsouza *et al*., 2017).

K. pneumoniae utilizes a variety of virulence factors, especially capsule polysaccharides(Arena *et al*., 2017). Lipopolysacchride and thick capsular polysaccharide layers are immunologically described as somatic (O-antigen) and capsular antigens (K-antigen), respectively that give mucoid colony on culture media(Compain *et al*., 2014). Inhibition

phagocytosis and hinder work of complement and attachment with cell surface (Huynh *et al.*, 2017).

Multiple genes responsible for forming capsular polysaccharide, so the difference in number of genes leading to difference in types of capsule (Baym *et al*., 2016). Adhesions fimbria genes, Siderophores and irobactin consider important virulence factors for bacteria, which are used for survival and immune evasion during infection (Paczosa &Mecsas, 2016). *K. pneumoniae* is accounts within the eight most commonly isolated pathogens in hospitals (Fu *et al*., 2018). The common use of antibiotic has failed to eradicate bacterial infections and has contributed towards the emergence of highly drug resistant bacterial strains(Jamil *et al*.,2014). The emergence of such bacterial strains results from chromosomal mutations and acquisition of multidrug resistance plasmids that encode for extended spectrum β-lactamases (ESBLs) (Khalid *et al*., 2017).

Extended spectrum beta lactamase are bacterial enzymes have ability to cause resistance against various types of β-lactam antibiotics, including third-generation cephalosporins and monobactams, though inhibited by clavulanic acid, inactivate all penicillins, amikacin, carbapeneme monobactams (aztreonam), and cephalosporins (ceftriaxone, cefotaxime, and ceftazidime) (Levinson, 2016). Available data suggest that the incidence of ESBL-producing *K. pneumoniae* has increased around the world(23%) in the United States, 4% in Canada, and 5% in Australia (Gelband *et al.*, 2015). More than 400 ESBL enzymes have been identified, and most of them have evolved due to mutations in the active center of the classic plasmid β-lactamases, including TEM-1, TEM-2, and SHV-1, with over 150 members. The CTX-M enzymes are the second largest group of ESBLs (Bajpai *et al.,* 2017). The majority of

ESBLs (mainly TEM and SHV type) were isolated from *K. pneumoniae* strains involved in nosocomial outbreaks (Liakopoulos *et al.,* 2016). Infections caused by ESBL-producing bacteria have increasingly subjected to therapeutic limitations, and patients with these infections are at high risk for treatment failure, long hospital stays, high health care costs, and high mortality (Diagbouga *et al.,* 2016).

The clinical bacterial isolates all over the world vary in the presence of ESBL enzymes and their pattern is changing rapidly over time (Bischoff *et al.,* 2018). Owing to the serious problems in the treatment of ESBL-producing *K. pneumoniae* infections, identifying the most prevalent ESBL enzymes locally is of great importance for countries, to monitor the changing of antibiotic resistance patterns (Al-Garawyi, 2016). These observations afford valuable information on ESBL epidemiology and could aid medical personnel in choosing the right and most effective treatment (Zhang *et al.,* 2016).

There are many microorganisms that could potentially function as probiotic, of which *Lactobacillus.* Probiotics are live microorganisms thought to be beneficial to the host organism (Jang *et al.,* 2019). According to the currently adopted definition by FAO/WHO, probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host (Mahdhi *et al*., 2017).

Probiotics affect the host beneficially, which may be direct or indirect, including enhanced barrier function, modulation of the mucosal immune system, production of antimicrobial agents, enhancement of digestion and absorption of food and alteration of the intestinal micro flora (Qinxiang *et al*., 2018; Maria *et al*., 2020).

The effective performance of the probiotic depends on their strong adherence and colonization of the human gut, which in turn improves the host immune system (Mousavi *et al*., 2020). Once the probiotic adheres to the cell, various biological activities take place, which primarily include the release of cytokines and chemokines. These then exert their secondary activity such as stimulation of mucosal and systemic host immunity (Goudarzvand *et al*., 2016; Tarrah *et al*., 2019).

Aim of the study:

This study was aimed to evaluate the hyper virulent isolates of *K. pneumoniae* isolated from urinary tract infections according to their antibiotic resistance and constituents of virulence genes, and studying the synergistic effect of probiotic against these isolates, as alternative antibacterial therapy *in vitro* and *in vivo* as an active or supportive treatment. The aim was fulfield by the following steps included:-

1. Collection of urine samples from patients suffering from urinary tract infections.

2. Isolation and identification of *Klebsiella pneumoniae* isolates by bacteriological culture, morphological examination ,biochemical and comfortable by Vetik-2 compact system.

3. Detection of the antibiotic resistance of bacterial isolates to different antibiotics and selection of the multidrug resistance isolates of the most drug of choice antibiotics.

4. Phenotypic detection of some virulence vactors(Capsule ability, adherence, biofilm formation, hemolysin,aerobactin and β- Lactam enzymes).

5. Molecular detection of virulent genes β- Lactames genes (*blaCTX* and *blaSHV*) in the selected isolates and some virulence factors genes (*K2A, fimH, mrkDV1, mrkDV2, hly, iucC, rmpA* and *magA*).

6. Studying the synergistic effect of probiotic against the hyper virulent isolates, *in vitro* and *in vivo*.

في 17(56.7%) وظهرت الجينات المشفرةلانزيمات البيتالاكتام (*blaSHV , blaCTX*) في جميع عزلات بكتريا *K.pneumoniae* (100%) , بينما كانت جميع العزلات خالية من الجينات التنظيمية للمخاطية *magA* و *rmpA* . كما بينت النتائج الى ان جينات *fimH* و *mrkDV* كانت الاكثر ترددا وفقا لانتشارهما في عزلات *K.pneumoniae*. وبينت النتائج ان الصفات المظهرية والوراثية لعامل ضراوة الكبسولة تتشابه مع 5(16.3%) من مجموع العزلات (30 عزلة) فقد ظهرت هذه العزلات سالبة لجينات *magA* و *rmpA*. كما اشارة نتائج التحري المظهري والوراثي عن صفة انتاج الهيمولايسين الى وجود تغاير بنسبة 93% و100% في جميع العزلات.حيث تم تحديد سبع جينات ضراوة من مجموع عشرة في كل من العزلة K6 و K4 . وتم تحديد العزلات الاكثر ضراوة (K26, K21, K19, K11) على اساس انتشار جينات الضراوة *iucC*و *K2A* في اغلب العزلات البكتيرية.

اظهرت نتائج الفعالية ضد المايكروبية للمعزز الحيوي تجاه عزلات بكتري *K. pneumoniae* الى وجود تأثير واضح على معظم العزلات متعددة المقاومة وعالية الضراوة, وبينت النتائج التاثير التازري الواضح لاستخدام مزيج المعزز الحيوي مع المضادات المنتخبة تجاه معظم العزلات غالبة الضراوة (K6 وK4) والعزلات عالية الضراوة (K11, K19 ,K21 وK 26) لبكتريا *K. pneumoniae* والذي كان اكثر تأثير من استخدام المعزز الحيوي او المضاد كلا على انفراد. فقد اظهر مزيج المعزز الحيوي بتركيز 100% مع مضاد Ceftazidime تأثيرا تازريا ضد عزلات *K. pneumoniae* المختارة اكبر من تأثير استخدام المكونين بشكل منفرد. كما اظهر استخدام مزيج المعزز الحيوي بتركيز 100% مع مضاد Ceftazidime تجاه عزلات *K. pneumoniae* K4, K6, K11, K19, K21 K26 تاثيرا تازريا مثبطا واضح اكر من استخدام المعزز الحيوي او المضاد بشكل منفرد على اساس اقطار مناطق التثبيط في حين لم يظهر استخدام