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Molecular Investigation of *mrk*A in *Klebsiella pneumoniae* Isolated from Nosocomial and Community Infection in Diyala Province, Iraq

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Abstract

Klebsiella pneumoniae is a pathogen relevant to hospital acquired and community acquired such as urinary tract infections, pneumonia, and wound and burn. It has high levels of antibiotic resistance and capability to produce biofilm. This study included comparing among six antibiotic resistance patterns for the hospital and community acquired infections, and detected biofilm gene (mrkA). Clinical samples were collected from Teach Bagubah Hospital, Al-Batool Maternity and Children Teaching Hospital, and private laboratories in Baqubah/Diyala, Iraq. To investigate antibiotics susceptibility, the Kirby-Bauer disk diffusion method was used to compare between community acquired isolates and hospital acquired isolates. Hospital acquired of K. pneumoniae were less susceptible than community acquired of K. pneumoniae isolates. Both isolates community acquired and hospital acquired were high rates of resistance for β-lactam/β -lactamase (Amoxicillin-clavulanate and Ticarcillin- clavulante). Screening and confirmation based on ESBL chromogenic agar are more sensitive than ESBL producers of the double disk synergy test, which were 30% and 60% produce- ESBLs in nosocomial acquired, and 20% and 40% in community acquired, respectively. The results of gel electrophoresis for PCR product using specific primers for mrkA gene prevalence were 83.33% and 66.6% in

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nosocomial and community acquired infection, respectively. The results are demonstrated more in the rest of the paper.

Keyword: K. pneumoniae, antibiotic resistance pattern, ESBL Producers, biofilm gene (mrkA).

التحري الجزيئي لجين mrkA في الكلبسيلا الرئويه المعزوله من التهابات المستشفيات والالتهابات المجتمعية في ديالى العراق

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الخلاصه

تعد الكلبسيلا الرئوية من اهم المسببات المرضيه ذات الصله باصابات المستشفيات والاصابات المجتمعية وتتضمن التهابات المجاري البولية والالتهابات الرئويه وتسبب تلوث الحروق والجروح وتمتلك قدرة عالية لمقاومه المضادات الحياتية وكذلك لها القدرة على تكوين الاغشيه الحيويه، وتضمن هذا البحث دراسه المظهرية لنمط المقاومه للمضادات الحيويه الستة المختارة في الدراسه لكلا عينات المستشفيات والعينات المجتمعية، وكذلك الكشف الجيني لجين (mrkA). تم جمع العينات السريريه من مستشفى بعقوبه التعليمي ومستشفى البتول والمختبرات الخارجيه في بعقوبه /ديالى العراق.تم استخدام طريقه Kirby-Bauerللانتشار القرصي لفحص الحساسيه للمضادات الحياتيه ومقارنة النتائج للعزلات الكلبسيلا المعزوله من المستشفيات والعزلات المجتمعية. تعد عزلات الكلبسيلا الرئويه من المستشفيات اقل حساسيه من العزلات المجتمعية . وتعد مجموعه المضادات الحياتيه β-lactam/β –lactamase (اموكلسين كلوفينيت و تاكارسلين كلوفينيت) اكثر مقاومه من بقيه المضادات. وتعد طريقه الكشف المظهري لعزلات الكلبسيلا الرئويه لانتاج انزيم البتالاكتام واسعه الطيف باستخدام وسط كورموجنك حساسة اكثر من طريقه الانتشار التازري للمضادات الحياتية اذ كانت النتائج 30% و 60% حساسيه الفحص في عزلات المستشفيات بينما 20% و40% لعزلات المجتمع. نتائج الترحيل الكهربائي باستخدام جل الترحيل نواتج تفاعل البلمرة لبرايمرات خاصه لجين mrkA كانت نسبة تواجد 83.33% و 66.6 % في عزلات المستشفيات والعزلات المجتمعية على التوالي.

الكلمات المفتاحية: الكلبسيلا الرئوية، نسق المقاومه للمضادات الحياتية، انتاج انزيم البيتالاكتام، جين الاغشيه الحيويه *mrk*A.

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Introduction

Klebsiella pneumoniae is a pathogen in the hospital acquired infection, and it might induce severe community acquired infections (CAIs), which are not considered opportunistic. However; the healthy patients do not share the risk factors for HAIs [1].

The community acquired infections are represented by various species from *Klebsiella* genus, with different profiles of antibiotics resistance and heterogeneous virulence factors. Nonetheless, this bacterium genus has been shown to acquire plasmid-borne antibiotic resistance in conjunction with virulence characteristics, posing a major threat to the Mexican population [2]. *K. pneumoniae* is a multidrug-resistant (MDR) bacteria that harms children and is a leading cause of hospital infections with significant morbidity and mortality due to restricted treatment options [3]. *K.* pneumoniae is a prevalent cause of nosocomial infections. It is resistant to carbapenems which is becoming more widespread all over the world. *K. pneumoniae's* ability to produce biofilms complicates patient treatment even further. The link between biofilm growth and patient prognosis, as well as carbapenem resistance, is yet unknown [4].

In 2020 [5], *K. pneumoniae* is a gram-negative opportunist bacterium that causes a variety of illnesses, including urinary tract infections, bacteremia, pneumonia, and liver abscesses. The rapid geographic distribution of the emergence of multidrug-resistant (MDR) and hypervirulent *K. pneumoniae* (hvKP) strains is especially worrying. Although, the specific mechanisms of pathogenicity and antibiotic resistance in *K. pneumoniae* remains unclearly.

The capacity to produce β-lactamase in the presence of β-lactam drugs, as well as a lipopolysaccharide and phagocytosis-resistant capsule, distinguishes these bacteria. A significant component for these bacteria is the creation of biofilm, which is largely responsible for persistent infections owing to their resistance to phagocytosis and death due to humoral and cellular immunity clear [3].

The capacity of *K. pneumoniae* to remain in the urinary system, as well as their connection to the *Fim*A, *fim*H, *mrk*A, and *mrk*D genes, which are involved in the creation of biological membranes. They were able to create biofilms using the tissue culture plate method [6]. Type 3 fimbriae are spiral-like threads that are encrypted by the *mrk*ABCD gene cluster and can be found on chromosomes or plasmids. They can also be gathered via the chaperone/usher pathway. The major structural component (*mrk* A) and the little adhesion tip (*mrk* D) are included in these genes, while *mrk* B, C, and E are involved in the gathering and control of fimbriae production, and *mrk*F is involved in the fimbrial surface constancy [7].

Aim of study compare between the antibiotic resistance pattern community-acquired infection and hospital-acquired infections for K. pneumoniae isolates from deferent clinical case as well as detectioning biofilm gene (mrk A).

Materials and Methods

Samples collection

Reactivation of two hundreds of *k. pnumoniae* according to Versalovic and his friend [8], that isolate from deferent clinical cases (urine, sputum, and swabs wounds and burns) in variety of hospitals and laboratories in Baqubah/Diyala, Iraq, From September 2021 to March 2022.

Antibiotic Susceptibility *K. pneumoniae*

The susceptibility of bacterial isolates to six antibiotics were investigated using the standard disc diffusion method according to Weinstein, Melvin P, and Lewis, James S. [9].

Phenotypic Detection of β-Lactamase production

1- Screening and Confirmation of ESBL Producers of the double-disk synergy test [10].
2- Screening and Confirmation Based on ESBL Chromogenic Agar [11].

Genotyping study of *K. pneumonia* DNA Extracts

Genomic DNA was extracted from bacterial growth using the gene aid extraction procedure. PCR was used to identify biofilm-encoding genes like *mrk*A. The sequences of oligonucleotide primers used in this study (from Macrogen, Korea)). *mrk*A-F(5`-ACGTCTCTAACTGCCAGGC-3`), *mrk* A-R (5`-TAGCCCTG TTGTTTGCTGGT-3`) for annealing temp 55℃ and product size 115bp [12].

Results and Discussion

Antibiotics susceptibility of *K. pneumoniae*

To determine the possible resistance of *K.Pneumoniae* isolates to six antibiotics from various classes. Twenty *K. pneumoniae* were tested using the Clinical and Laboratory Standards Institute's antibiogram method (CLSI-2020) [9]. This done through using disc diffusion method (Kirby-Bauer method) against six classes of antibiotics. The results Table (1), indicate 10 of *K. pneumoniae* isolates nosocomially acquired infection, For β-lactam/β -lactamase inhibitor combination agents Ticarcillin-clavulante (TIM), and Amoxicillin-clavulanate (AMG), moderate resistance to the third generation cephalosporins Ceftriaxone (CRO), and carbopemes Imipenem (IMP), and less resistance to fluoroquinolones Levofloxacin (LEV). This result is closer to Al–Zubaidi, and Al–Taai, (2020) [13] which species high resistance against β –Lactam groups, and less other classes of antibiotic Aminoglycosides, and fluoroquinolones. Furthermore, our results agreed with Mirzaie and Ranjbar (2021)[14]. *K. pneumoniae* is an important multi-drug-resistant Gram-negative pathogen, associated with nosocomial [15]. Carbapenems are the drugs of choice for many infections caused by *E. coli* and *K. pneumonia*e [16].

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Table 1: Antibiotic Patterns of resistance of *K. pneumonia*e for nosocomial acquire infection

Name	Patterns of resistance	No. of isolates
R1	TIM, AMG, IMP MDR	1
R2	TIM, AMG, CRO MDR	1
R3	TIM, AMG, CRO, IMP, LEV MDR	1
R4	TIM, AMG, CRO, IMP MDR	1
R5	TIM, AMG, CRO, IMP, AK XDR	1
R6	TIM, AMG, IMP, LEV, AK MDR	1
R7	TIM, AMG, LEV, AK XDR	1
R8	TIM, AMG, CRO, IMP, LEV, AK PDR	2
R9	TIM, AMG, CRO, IMP XDR	1

Results indicated in table (2) antibiogram results of 10 *K. pneumonia*e isolates community acquired infection,heighest resistance For β-lactam/β -lactamase inhibitor combination TIM, and AMG, cephalosporins Ceftriaxone (CRO),while moderate resistance to the third-generation fluoroquinolones (LEV), and aminoglycoside(AK).

Table 2: Antibiotic resistance patterns of *K. pneumonia*e for community acquire infection

NO. of isolates	Resistance patterns	Pattern name
1	TIM, LEV, AK, CRO XDR	R1
2	TIM, AMG, CRO IMP XDR	R2
1	TIM, AMG ,CRO MDR	R3
2	TIM, AMG, CRO ,LEV MDR	R4
1	TIM, AMG, CRO IMP MDR	R5
1	TIM, AMG, CAZ, AK XDR	R6
2	TIM,AMG, CRO IMP,LEV, AK PDR	R7

For comparing between community-acquired isolates and hospital-acquired *K. pneumoniae* isolates, the statistic significantly (*p. value* <0.05) has been used in this investigation. Hospital-acquired *K. pneumoniae* are less sensitive to antibiotics than community-acquired K. pneumoniae. Both community-acquired and hospital-acquired isolates the highest rates of resistance for β-lactam/β -lactamase (AMG, and TIM). It is agreed with Liu and researcher's study in (2020)[17], resistance rates for *K. pneumoniae* are lowest in community-acquired infections and greatest in nosocomial infections. However, other variables such as antibiotic

usage might potentially contribute to antimicrobial resistance. This disparity might be attributable to the various populations' infection types or geographical variances in *K. pneumoniae* resistance features. The high prevalence of antibiotic resistance and virulence genes in conjunction with a significant relationship between the strains revealed a high pathogenic capacity of the isolated pathotypes of *K. pneumoniae* [18].

Multi-drug Resistance of *K. pneumoniae* Isolates

According to Weinstein, Melvin P, & Lewis, James S. (2020) [9] that classified to multi-drug resistance (MDR), extended drug resistance (XDR), and pan-drug resistance (PDR) were studied in 20 *K. pneumonia*e. MDR is now defined as follows: 50%, and 40% of isolates in nosocomial acquired infection and community acquired infection were confirmed as MDR. The most common agents involved in MDR were β-lactam/β-lactamase inhibitor combination (TIM and (AMG), carbapenems (MEM). Among these isolates, 30%, and 40% were resistant to four or five classes of antibiotics assembly criteria for XDR organisms. Moreover, 20%, and 20% of isolates were resistant to all antibiotic classes (six), and hence considered as PDR isolates in nosocomial acquired infection and community acquired infection, this result were non-significantly (*p. value* > 0.05), this result agree with Le [19]. The biggest percentage of MDR isolates (53.1%) were from nosocomial infections, followed by isolates from community infections (23.3%). Infection control and empirical treatment practices MDR, XDR, and PDR strains of *K. pneumoniae* have been identified as one of the most serious dangers to public health. PDR *K. pneumoniae* infections are usually related with nosocomial infections (the high prevalence of the usage of antibiotics in Diyala hospitals is well regulated).

Phenotypic Detection of β-Lactamase production

A: Screening and Confirmation of ESBL Producers the double-disk synergy test.

The carbapenem-resistant isolates were screened for ESBL formation using ceftazidime, cefotaxime, ceftriaxone, and azetreonam (30 g each) antibiotic disks in a disk diffusion

technique. According to the CLSI (2020). The results of ESBLs producing isolates by disk diffusion are shown in Figure (1), which are 3(30%), and 2(20%) isolates produce ESBLs enzyme while 7(70%), and 8(80%) of the isolate are non ESBLs enzyme producer in nosocomial acquire and community acquired.

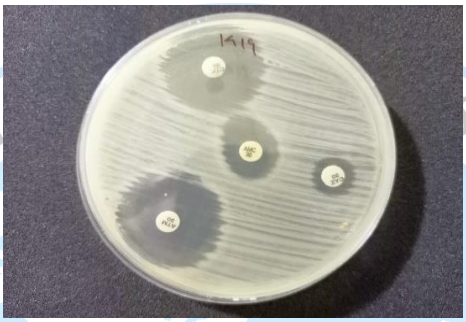

Figure 1: Phenotypic screening for ESβLs production.

B: Screening and Confirmation Based on ESBL Chromogenic Agar

The incidence of ESBLs producing *K.pneumoniae* is dark blue colonies as shown in Figure (2), 6(60%), and 4(40%) isolates produce ESBLs enzyme while 4(40%), and 6(60%) of the isolate are non ESBLs enzyme producer in nosocomial acquired and community acquired.

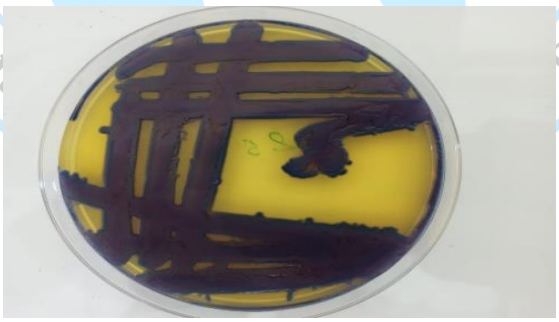

Figure 2: ESBL Chromogenic Agar show *K.pneumoniae*

When campier result of nosocomial acquired and community acquired (A) method and (B) method, the highest sensitive test of *K. pneumoniae* produce- ESBLs was in method B that 30%,

and 60% produce- ESBLs in nosocomial acquired,20%,and 40% in community acquired. Chromogenic Agar are more specific than ESBL Producers the double-disk synergy test. Peptones and growth factors supply nitrogen, vitamins, minerals, and amino acids, all of which are necessary for growth. ESBL-producing microbes can be identified using a chromogenic combination. All non-ESBL-producing bacteria are inhibited by the supplement (ceftazidime 1.50 mg, cefotaxime 1.50mg , ceftriazone 1.00mg, Aztreonam 1.00mg, fluconazole 5.00 mg additional to 500ml chromogenic media [11].

The phenotypic and genetic features of *Klebsiella* isolates recovered from healthy and unwell babies showed no change [20] The development and spread of ESBL among Gram-negative bacteria and possible horizontal transfer calls for concern, especially in view of treatment failure, high treatment cost, and consequent discomfort to patients. The early detection of ESBL-producing bacteria carriers is essential to minimize their impact and spread [11].

Molecular study of *K. pneumoniae* DNA.

For complete genomic DNA recovered from K. pneumoniae isolates, a genomic DNA purification kit (Promega, USA) was used. Extraction genomic DNA from 10 PDR K. pneumoniae isolates were validated qualitatively as bands by gel electrophoresis.

Molecular detection of biofilm gene (*mrk*A) by PCR in *k. pneumoiae*, these 9 PDR clinical isolates form 6 *k.pneumoiae* the nosocomial acquired infection and 3 *k. pneumoiae* the community acquired infections.

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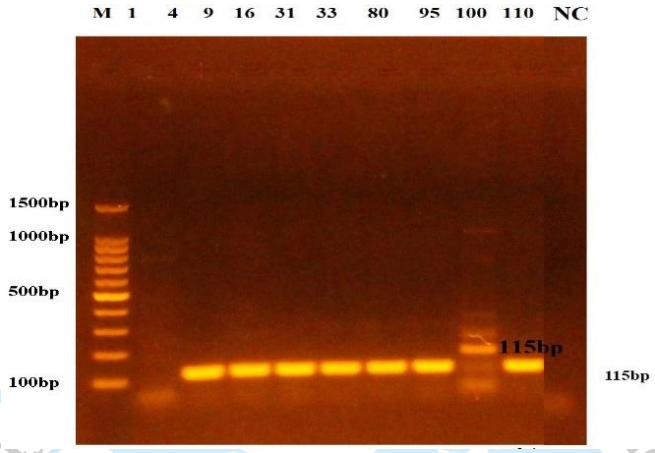

Figure 3: Amplification results of *mrk*A primers in *Klebsiella pneumoniae* samples fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker, NC: negative control. The electric current at 100 volts for 60min.

The results of gel electrophoresis for PCR product by specific primers for this gene are shown in Figure (3), which are 83.33%, and 66.6% biofilm gene (mrkA) appeared in nosocomial acquired infection and community acquired infection, respectively. However; clinical isolates (k.1 and k.95) did not appear mrkA gene. The results were the closest to Mirzaie and Ranjbar [14]. All clinical isolates have the same 88% frequency of biofilm-associated genes such mrkA, fimH, and mrkD. The expression of the mrkA gene is positively linked with the buildup of biofilm biomass in a correlation study [21].

Conclusion

Biofilm gene *mrk*A is important for antimicrobial resistance, when comparing between community-acquired isolates and hospital-acquired isolates. *K. pneumoniae* is resistance for antibiotic. Hospital-acquired isolates of *K. pneumoniae* are less susceptible than community-isolates. Both community-acquired and hospital isolates are high rates of resistance for β-lactam/β -lactamase (AMG, and TIM). ESBL Chromogenic Agar are sensitive more than ESBL Producers the double-disk synergy test.

References

1. B. L. Meatherall, D. Gregson, T. Ross, J. D. Pitout, K. B. Laupland, The American journal of medicine, 122(9), 866-873(2009)
2. U. Garza-Ramos, H. Barrios-Camacho, S. Moreno-DomÝnguez, J. Toribio-JimÚnez, D Jardˇn-Pineda, J. Cuevas-Pe, A. Romßn-Romßn, New microbes and new infections, 23, 17-27(2018)
3. S. A. Jasim, S. A. Abdulrazzaq, R. O. Saleh, Systematic Reviews in Pharmacy, 11(6), (2020)
4. D. Ragupathi, N. Kumar, M. Sethuvel, D. Prabaa, T. Dwarakanathan, H. Murugan, D. Umashankar, Y. Monk, N. Peter, V. Balaji, Frontiers in Microbiology, 11(3220), (2020)
5. W. Guoying, Z. Guo, C. Xiaoyu, X. Longxiang, W. Hongju, International Journal of Environmental Research and Public Health, 17(17), 6278(2020)
6. A. H. Alwan, N. N. Khwen, Al Mustansiriyah Journal of Pharmaceutical Sciences, 17(1), 12-12(2017)
7. P. K. Michelle, M. Joan, Microbiology and Molecular Biology Reviews, 80(3), 629-661(2016)
8. J. Versalovic, K. Carroll, G. Funke, J. Jorgensen, M. Landry, D. Warnock, Manual of Clinical Microbiology. 10th ed., (American Society of Microbiology, 2011)
9. W. P. Melvin, L. S. James, Journal of clinical microbiology, 58(3), e01864-01819(2020)
10. H. Romney, B. M. April, H. A. Janet, S. N. Audrey, Journal of clinical microbiology, 59(12), e00213-00221(2021)
11. M. Osterblad, J. Kirveskari, S. Koskela, P. Tissari, K. Vuorenoja, A. J. Hakanen, J. Jalava, Euro Surveill, *14*.(40), (2009)
12. C. Vuotto, F. Longo, C. Pascolini, G. Donelli, MP. Balice, MF. Libori, PE. Varaldo, Journal of applied microbiology, 123(4), 1003-1018(2017)

13. S. J. J. Al–Zubaidi, H. R. R. Al–Taai, International Journal of Psychosocial Rehabilitation, 24(05), (2020)

14. M. Amir, R. Reza, AMB Express, 11(1), 1-11(2021)

15. B. M. J. AL-Muqdadi, Molecular Typing and KPC enzyme Detection of *Klebsiella Pneumoniae* Isolated from Some Clinical Samples of Baghdad, M.Sc. Thesis,, University of Baghdad(2019)

16. Y.S. Huang, W. C. Tsai, J. J. Li, P. Y. Chen, J. T. Wang, Y. T. Chen, S. C. Chang, Scientific reports, 11(1), 1-10(2021)

17. C. Liu, P. Du, N. Xiao, F. Ji, T. A. Russo, J. Guo, Virulence, 11(1), 1215-1224(2020)

18. M. Ahmadi, R. Ranjbar, P. Behzadi, T. Mohammadian, Expert Review of Anti-infective Therapy, 20(3), 463-472(2022)

19. T. Le, L. Wang, C. Zeng, L. Fu, Z. Liu, J. Hu, Antimicrob Resist Infect Control, 10(1), 41(2021)

20. L. Zeng, C. Yang, J. Zhang, K. Hu, J. Zou, J. Li, X. Zhang, Front Cell Infect Microbiol, 11, 656070(2021)

21. W. Zhibin, Z. Ding, Z. Li, Y. Ding, F. Jiang, J. Liu, Microbial Pathogenesis, 159, 105121(2021)