Phenotypic Expression of Type 1 *Klebsiella pneumoniae* Fimbriae in *Escherichia coli* HB101

التعبير المظهري لخمل النمط الأول لبكتريا *Klebsiella pneumoniae* في بكتريا *Escherichia coli* HB101

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Abstract

Four selected isolates of *K. pneumoniae* subsp. *pneumoniae* were tested for adhesion and the results revealed that isolates MK20, MK1, MK2, MK6 displayed adhesive capability with means (76.25 ± 6.0),(75.88 ± 5.13), (69.83 ± 8.43), (27.4±4.65) bacteria/ epithelial cell, respectively. Agarose gel electrophoresis was performed to detect plasmid profile for the four selected isolates. Results showed that all isolates exhibited two bands of small plasmid DNA with molecular size ranged from less than 4kb for the smaller one to more than 12kb for the larger one as compared with DNA molecular size marker (lambda phage restricted with *EcoR*I & *Hind*III). Besides two bands of large plasmid DNA were obtained from the isolates MK20, MK1, MK6, but the isolate MK2 didn't show any band of large plasmid DNA. Transformation of plasmid DNA was performed using standard genetically modified *E. coli* HB101 strain as the recipient for plasmids of the selected isolates, while *E. coli* HB101with pBR322 plasmid was used as a positive control. In respect to the adhesion test, it was observed that the transformants, designated as (T1, T2, T6, T20), showed adherence capability of: (54.37±5.38), (48.33±4.475), (43.46±0.625), (33.0±6.71) for the transformants T2, T20, T1, T6, respectively. However, these transformants showed no significant differences in the adherence capability, however, the mean of adhesion capabilities of the transformants were less than that of the wild type isolates.

المستخلص

تم التحري عن قابلية التصاق اربع عزلات تابعة لبكتريا *K.pneumoniae* subsp. *pneumoniae* وقد بينت النتائج بأن القابلية الالتصاقية للعزلات MK20 ، MK1 ، MK2 ، MK6 كانت بمتوسط (76.25 ± 6.0) ، (75.88 ± 5.13) ، (69.83 ± 8.43) ، (27.4 ±4.65) خلية بكتيرية ملتصقة / خلية طلائية على التوالي . تم القيام بالتحري عن المحتوى البلازميدي للعزلات الأربعة وقد اظهرت النتائج امتلاك جميع العزلات لحزمتين بلازميديتين صغيرتين اذ قدرت احجامها بين اقل من Kb4 زوج قاعدي لصغراهما و اكبر من Kb 12 زوج قاعدي للحزمة الاكبر باستخدام دنا العاثي lambda مقطع بكل من إنزيمي التقييد I *EcoR* و *Hind*III كدليل حجمي ،فضلا عن احتواء ثلاث عزلات على دنا حزمتين بلازميديتين كبيرتين .تم اجراء تجربة التحول وذلك بتحويل السلالة القياسية *E. coli* HB101 ألمحورة وراثيا الخالية من البلازميدات والحساسة لجميع المضادات الحيوية وتأهليها لإستقبال بلازميدات عزلات *K.pneumoniae* المنتخبة فضلا عن تحويلها ببلازميد pBR322 كسيطرة موجبة . لقد أظهرت النتائج بأن أعلى نسبة تحول كانت لمضاد الكلورامفينيكول لجميع العزلات المتحولة حيث كان معدل تكرار التحول لهذا المضاد(2.91×10^{-6}) ، اما أقل

تكرار تحول للعزلات المتحولة كان لمضاد السيفالوثين بمعدل (0.33×10^{-6}). بالإضافة الى ذلك فقد تم اختبار قابلية المتحولات على إلإلتصاق على الخلايا الطلائية المأخوذة من تجويف الفم لأشخاص أصحاء ، إذ بلغت متوسطات إلإلتصاق للخلايا المتحولة والتي اطلق عليها T1,T2,T6, T20: (54.37 ± 5.38) ، (48.33 ± 4.475) ، (43.46 ± 0.625) ، (33.0 ± 6.71) للمتحولات T2، T20، T1، T6 على التوالي ، ولم يلاحظ وجود فرق معنوي بين قابلية الإلتصاق لتلك المتحولات وقد لوحظ بأن متوسط التصاق المتحولات كان اقل من متوسط التصاق العزلات الأصلية .

Introduction

Type 1 pili adhesion organelles mostly predominance among *Klebsiella pneumoniae* [1] it is, responsible for the adherence of the bacteria to intestine, urogenital and respiratory tracts of human and animal [2, 3, 4].This considered first step which play a critical role in bacterial pathogenesis by allowing bacteria to attach and colonize specifically to host cell surface and subsequently initiate a disease [5]. It was observed that type 1 pili of *K. pneumoniae* mediate the attachment of these bacteria to bladder epithelial tissue causing cystitis [6].

It was found that the adhesion capability of *K. pneumoniae* is plasmid – encoded and they noticed that when this plasmid transformed to *E. coli* K12 strain, the adhesion indices raised two – fold than that the indices of the wild type *K. pneumoniae* isolates [7]. In many studies [7, 8] found that the genes encoding for pili mediated adhesion of *K. pneumoniae* strain to the intestinal epithelium and a non fimbrial protein CF29 were located on conjugative plasmid called "R – Plasmid " with molecular size approximately 180 kb .

Multiple gene products were observed to be necessary for the phenotypic expression of *K. pneumoniae* fimbriae; and the fimbrial expression is similar to that in *Escherichia coli*; and at least four genes constituted the fimbrial gene cluster of *K. pneumoniae* [9]. In cloning experiment it was found that *fim A* genes encoding type 1 major fimbrial subunits of two isolates of *K. pneumoniae* could be phenotypically expressed in *Escherichia coli* HB101 [3]. *K. pneumoniae fim H* gene is found in 90% of strains from various environmental and clinical sources. FimH is a minor component of type 1 pili that is required for the pili to bind and agglutinate guinea pig erythrocytes in a mannose-inhabitable manner [10].

Expression of type 1 fimbriae in *K. pneumoniae* was found to be phase variable. By use of a PCR-based assay, the orientation of the *fim* switch during colonization and infection was investigated and was found to be all “off” in the intestine and lungs but all “on” in the urinary tract. It was suggested that during colonization and infection, there is pronounced selective pressure in different host environments for selection of either the type1 fimbriated or nonfimbriated phenotype of *K. pneumoniae* [11].

The aim of the present study is to designate the variation in type 1 pili expression among the wild type isolates of *K. pneumoniae* from different sources in comparison with that that transformants.

Materials and Methods

Bacterial isolates and strain: four local isolates of *K. pneumoniae pneumoniae* and modified standard strain *E. coli* HB101 were used Table (1). All isolates and strain

were maintained in screw-caped universal tubes containing nutrient agar (Acumedia, U.S.A.) and stored at 4°C.

Table (1) :Isolates and standard bacterial strains

Bacteria	*Sources and characteristic of bacteria
MK1	UTI specimen With type-1 fimbriae
MK2	Wound specimen With type-1 fimbriae
MK6	Blood specimen With type-1 fimbriae
MK20	Sputum specimen With type-1 fimbriae
Modified standard strain E. coli Hb101	(1)(rec- , F- ,hsdR-, hsdM-), plasmidless with no adhesive ability
standard strain E. coli HB101	(2)With pBr322 (AMPr,TEr)

* The isolates and the standard strains are provided by [12]

(1) rec^- , F^-, $HsdR^-$, $HsdM^-$ = loss of recombination, loss of fertility factor, loss of restriction and modification systems respectively.

(2)Amp^r, Te^r = resistant to ampicillin and tetracycline respectively.

In vitro adhesion test: four isolates of *K.pneumoniae* subsp. *pneumoniae* were examined for adhesion test according to the method described by [13]. The epithelial cells were obtained from the buccal cavity of healthy people and the number of attached bacteria was calculated to each 20 epithelial cells.

Antibiotic selective medium: The medium was prepared according to method described by [14] and appropriate antibiotics Table (2) were added to the medium as selective markers.

Table(2): Stock and final concentrations of antibiotic solutions:

Types of Antibiotic Solutions	Concentration of the Stock Solution (mg/ml)	Final Concentration (µg/ml)
Amoxicillin	50	50
Cephalothin	50	50
Gentamicin	50	15
Streptomycin	50	15
Chloramphenicol	50	1024
Tetracycline	50	10

Isolation of Plasmid DNA (Alkaline Method): plasmid DNA of the four selected isolates was extracted using the method described by [14].

Agarose gel electrophoresis: Agarose gel was prepared using the method described by [14], this method included the addition of ethidium bromide at final concentration of 0.5µg / ml to the 0.7% melted agarose.

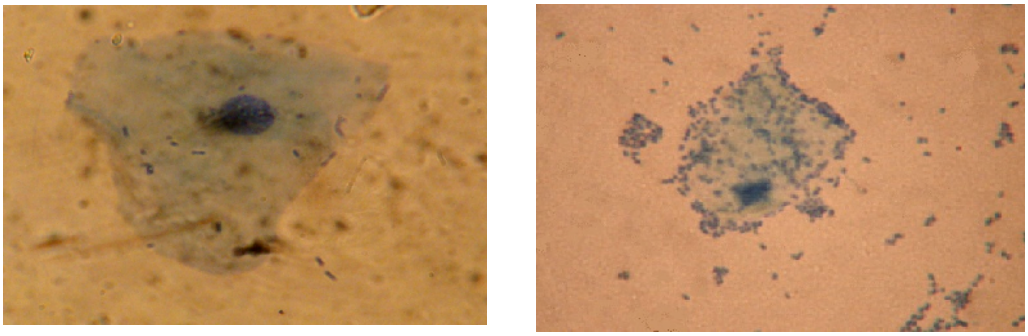
Transformation experiment: Modified standard strains of *E. coli* HB101 were transformed with plasmid DNA by the $CaCl_2$ [14].

Statistical Analysis: Bacterial adherence to 20 epithelial cells was statistically analyzed by one-way analysis of variance (ANOVA). Least significant difference (LSD) was used to determine the significant difference betwee

adherence capability. Paired-samples T- test was used to test the significance between the mean of adherence capability for the transformants and wild types isolates. The statistical package used to determine the above statistical parameters was "Statistical Package for Social Science (SPSS)" version 12.0 for windows (2000) [15].

Results and Discussion

Adhesion test: the isolates displayed differences in their adherence to human buccal cavity epithelial cells of healthy people. It was noticed that isolates MK20, MK1, MK2 displayed high adhesive capability with mean values of (76.25 ± 6.0), (75.88 ± 5.13), (69.83 ± 8.43) bacteria/ epithelial cell respectively, while the isolate MK6 displayed a lowest adhesive capability with mean value of (27.35 ± 4.65) bacteria/ epithelial cell. It was also found that there was no significant difference for adhesive capability among MK20, MK1, MK2 isolates Figure (1). There was variation in the adhesion capability of *K. pneumoniae* to epithelial cells of different animal species as indicated by the previous studies[6, 13, 16, 17]; besides it was observed that adhesive capacity is affected by many conditions such as growth phase, temperature, and incubation period [18].



A **B**

Fig(1): Adhesion capability of *K. pneumoniae* isolates
A: Epithelial cell control from human buccal cavity (100X).
B: *K. pneumoniae* MK20 isolate adhered to human buccal epithelial cell (100X).

Plasmid profile: Patterns of plasmid were studied. Results revealed that the four selected isolates (MK1, MK 2, MK 6, MK 20) have two bands of small plasmid; the smallest one with molecular size less than 4 kb, and the other plasmid band seemed to be more than 12kb as compared with standard DNA of the Lambda phage restricted with both *Eco*RI and *Hind*III as indicated in Figure (2), in addition to the presence of two mega plasmids in MK1, MK6, MK20 isolates , whereas the MK2 didn't show any mega plasmid and that may be attributed to the low DNA concentration or technical error during electrophoresis process Figure (2).

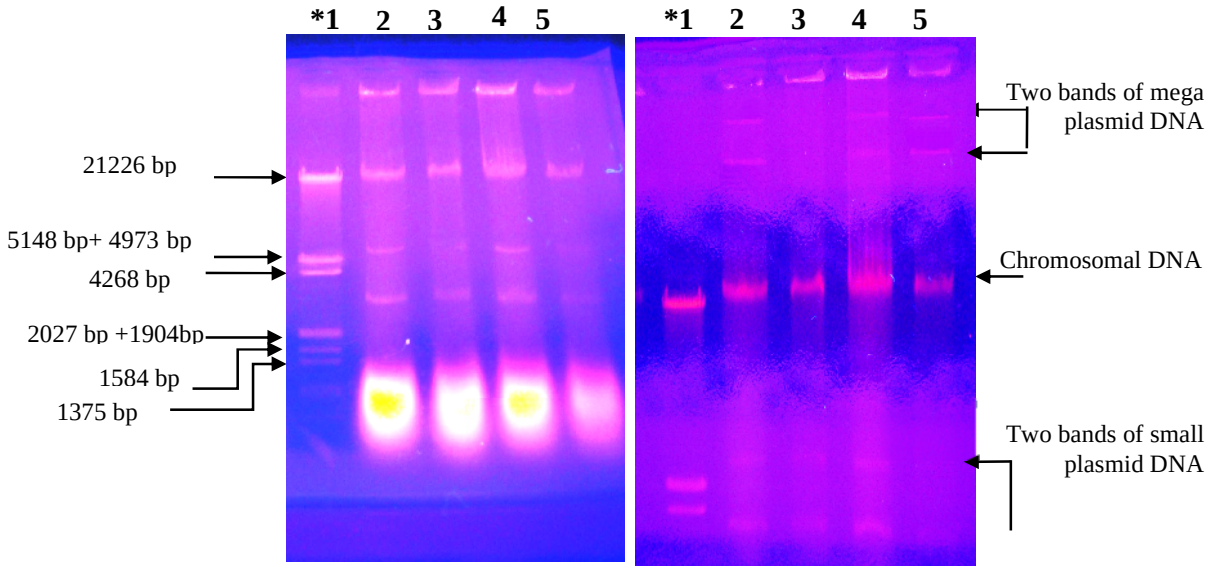


Fig (2):Agarose gel electrophoresis for DNA of *K. pneumoniae* isolates (agarose concentration was 0.7%).
A: Agarose gel electrophoresis for plasmid DNA of *K. pneumoniae* isolates with λ- phage DNA marker restricted with *EcoR*I & *Hind*III after one hour.
B: Agarose gel electrophoresis of *K. pneumoniae* with the presence of two mega plasmid DNA bands in Lanes 2, 4 and5 after one and half an hour.
Lane *1 represents DNA bands of λ - phage marker.
Lanes 2, 3, 4, and 5 represent MK6, MK2, MK1, and MK20 isolates respectively.

Transformation experiment: Transformation assay was performed so as to specify the role of plasmids in adhesion capability. Standard strain of *E. coli* HB101 was used as recipient host for the plasmids of *K. pneumoniae* isolates (MK20, MK1, MK2, MK6), besides pBR322 plasmid was used as positive control, and then the transformation mixture was spread onto antibiotic selective media so as to characterize the colonies that harbor the new plasmids and thus the new traits for antibiotic resistance. The results indicated that the highest transformation frequency with antibiotics was for chloramphenicol with average (2.91×10^{-6}), while the lowest transformation frequency was for cephalothin with average (0.33×10^{-6}). In order to detect whether the adhesion capability was transformed or not and whether this capability is plasmid encoded or not, some of these transformants were tested for adhesion capability Figure (3). Results showed adherence capability of: (54.37±5.38), (48.33±4.475), (43.46±0.625), (33.0±6.71) for the transformants T2, T20, T1, T6, respectively as indicated in Table (3).

Table (3): Adherence capability of the transformants

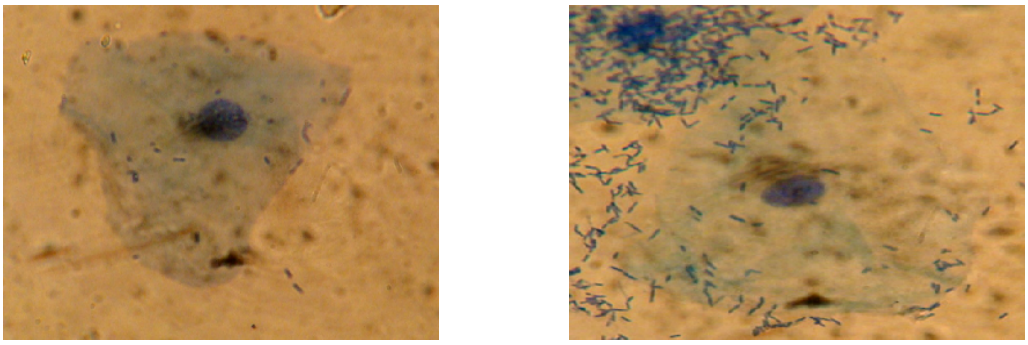
Transformant (T)	* Mean	** SEM
T2	54.37	5.38
T20	48.33	4.475
T1	43.46	0.625
T6	33.0	6.71

* **No significant difference (P >0.05)**
** **Each value is mean ± standard error for mean (n = 2)**

However, these transformants showed no significant differences in the adherence capability, however, the mean of adhesion capabilities of the transformants were less than that of the wild type isolates. Transformants of streptomycin and pBR322 transformants of tetracycline were then examined using agarose gel electrophoresis as the results demonstrated in Figure (4); from which it was obvious that the plasmids of the four selected isolates of *K. pneumoniae* , and pBR322 plasmid were transformed successfully but the large plasmid of wild type isolates was undetectable which may allow to conclude that not all genetic determinants have the ability to transform may be because of the size of the large plasmid and that explains the low transformation frequency of ß – lactam antibiotics or may be the genes encoding these antibiotics are weakly expressed in the recipient cell [19], or may be some genes that encode for some antibiotic resistance are chromosomally regulated [20,21,22] .

It was reported that the adhesion capability is plasmid – encoded and they found that when this plasmid transformed in *E. coli* K12, the adhesion capabilities raised two – fold more than those of the wild type *K. pneumoniae* isolates and they attributed that the low expression of CF29 surface adhesin of *K. pneumoniae* may related to the capsule that shields the function of this surface adhesion. Concerning the difference in the level of expression of this protein between *K. pneumoniae* isolates and the transconjugants, this may attributed to some genes encoding CF29 might not transfer to the transconjugants and that's why high adhesion indices found in the transconjugants rather than the wild type [7] .

Another study [8] reported that clinical isolates of *K. pneumoniae* exhibit CF29 surface adhesin and possess R – plasmid that harbors ß – lactam, aminoglycosides, and tetracycline antibiotics resistance, and when this plasmid transferred to *E. coli* K12 strain, high adhesion capabilities in the transconjugants were detected, whereas the isolates that didn't express this surface adhesin showed, very low adhesin capability. In comparison with another study [23] which found that the transfer of ESBL- encoding plasmid to standard strain of *E. coli* K12 didn't participate in the adhesion of these transconjugants to the epithelial cells.



A B

Fig (3): Adhesion of *E. coli* HB101 transformant to human buccal epithelial cell with epithelial cell control (100X)

A: Epithelial cell control from human buccal cavity.

B: Adhesion of *E. coli* HB101 transformant T20 to human buccal cavity epithelial cell.

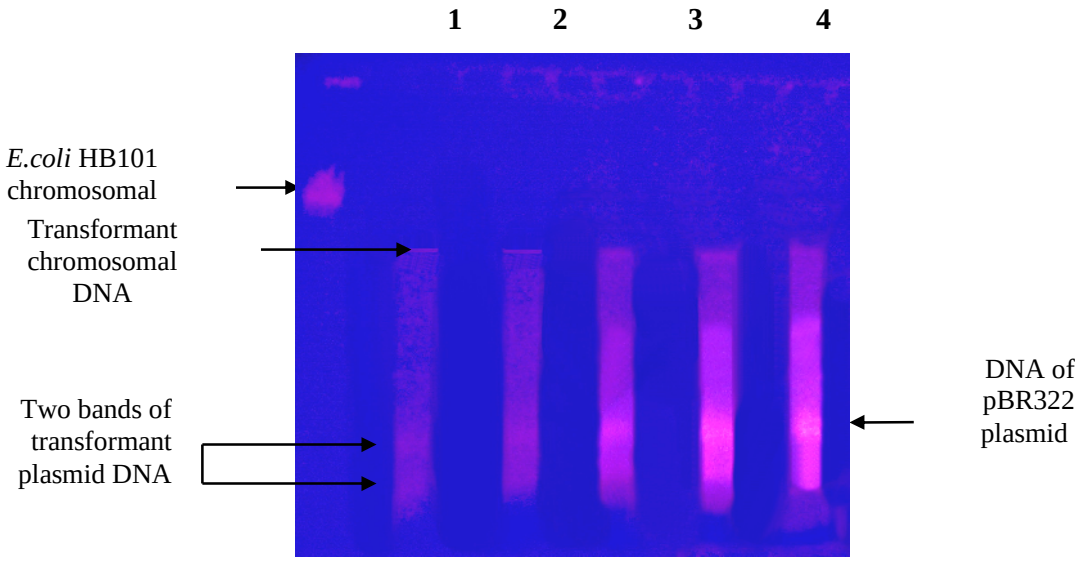


Fig (4): Agarose gel electrophoresis of the transformants with pBR322 plasmid (agarose concentration (0.7%), voltage 5volt/cm, during 2.5hrs).
Lanes 1, 2, 3, 4, 5, and 6 represent the modified *E. coli* HB101 standard strain, streptomycin transformants T20,T1, T2, T6, and pBR322 tetracycline transformant respectively.

References

1. Livrelli, V., De Champs, C., Di Martino, P.; Darfeuille – Michaud, A.D.; Forstier, C. and Joly, B. (1996). Adhesive properties and antibiotic resistance of *Klebsiella, Enterobacter,* and *Serratia* clinical isolates involved in nosocomial infections. J. Clin. Microbiol. 34(8): 1963 – 1969.
2. Fader, R.C.; Avots – Avots, A.A. and Davis, C.P. (1979). Evidence for pili – mediated adherence of *Klebsiella pneumoniae* to rat bladder epithelial cells *in vitro.* Infect. Immun. 25(2): 729 – 737.
3. Ayras, G. H.; Altman, L. C.; and Fretwell, M. D. (1982). Effect of decreased salivation and pH on the adherence of *Klebsiella* spp. to human buccal epithelial cell. Infect. Immun. 38(1): 179 – 182.
4. Gerlach, G.; Clegg, S.; Ness, N. J.; Swenson, D. L.; Allen, B.L.; and Nichols, W. A. (1989). Expression of Type 1 Fimbriae and Mannose-Sensitive Hemagglutinin by Recombinant Plasmids. Infect. Immun. 57(3):764-770.
5. Holt, S. C.; and Ledbetter, E. R.(1998). Relationships in prokaryotic cells. *In*: Topely & Wilson's Microbiology and Microbial Infection. Systematic Bacteriology. By: Collier, L.; Balows, A.; and Sussman, M. Vol. Two. 9th ed. Euston Road, London.
6. Fader, R.C.; Avots – Avots, A.A. and Davis, C.P. (1979). Evidence for pili – mediated adherence of *Klebsiella pneumoniae* to rat bladder epithelial cells *in vitro.* Infect. Immun. 25(2): 729 – 737.
7. Darfeuille – Michaud, A.D.; Jallat, C.; Aubel, D.; Sirot, D.; Rich; C. And Joly, B. (1992). R – Plasmid encoded adhesive factor in *Klebsiella pneumoniae* strains responsible for human nosocomial infection. Infect. Immun. 60(1): 44 – 55.
8. Di Martino, P.; Bertin, Y.; Girardeau, J.P.; Livrelli, V.; Joly, B. and Darfeuille – Michaud, A. (1995). Molecular characterization and adhesive Properties of CF29K,

an adhesion of *Klebsiella pneumoniae* strains involved in nosocomial infections. Infect. Immun. 63(11): 4336 – 4344.

9. Clegg, S.; Purcell, B. K.; and Pruckler, J. (1987). Characterization of Genes Encoding Type 1 Fimbriae of *Klebsiella pneumoniae, Salmonella typhimurium, and Serratia marcescens*. Infect. Immun. 55(2):281-287.

10. Stahlhut, S.G.; Chattopadhyay, S.; Struve, C.; Weissman, S.J., and Sokurenko, E.V. (2009). Population variability of Fim H type 1 fimbrial adhesion in *Klebsiella pneumoniae*. J. Bacteriol. 191(6): 1941-1950.

11. Struve, C.; Forestier, C.; and Krogfelt, K.A. (2003). Application of a novel multi – screening signature tagged mutagenesis assay for identification of *Klebsiella pneumoniae* genes essential in colonization and infection. Microbiology. 149: 167 – 176.

12. Al-Nasiri, M. A. (2009). Bacteriological and Genetic Study on Locally Isolated *Klebsiella pneumoniae.* M.sc. thesis / College of Science / Baghdad University.

13. Iwahi, T.; Abe, Y.; and Tsuchiya K. (1982). Virulence of *Escherichia coli* in ascending urinary tract infection in mice. J. Med. Microbiol. 15: 303 – 316.

14. Sambrook, J.; Fitgah, E.; and Maniatis, T. (2000). Molecular cloning: A laboratory Manual. 3rd ed. Cold spring, Harbor Laboratory, New York.

15. SPSS. (2000). Statistical Package for Social Sciences. Version 12 for windows. Chicago, U. S. A.

16. Hagberg, L.; Judal, U.; Korhonen, T.K.; Lidsin – Johnson, G.; Lindberg, U.; and Eden, C.S. (1981). Adhesion, Hemaglutination, and Virulence of *Escherichia coli* Causing Urinary Tract Infection. Infect. Immun. 31(2): 564- 570.

17. Iwahi, T.; Abe, Y.; Nakao, M.; Imada, A., and Tsuchiya K. (1983). Role of type 1 fimbriae in the pathogenesis of urinary tract infection induces by *Escherichia coli* in mice. Infect. Immun. 39(3): 1307 – 131.

18. Favre-Bonté, S.; Darfeuille – Michaud, A.; and Frostier, C. (1995). Aggregative adherence of *Klebsiella pneumoniae* to human Intestine – 407. Infect. Immun. 63(4): 1318 – 1328.

19. Jones, D.; and Sneath, P.H. A. (1970). Genetic Transfer and Bacterial Taxonomy. Biol. Rev. 43(1): 40 – 81.

20. Kaye, K. S.; Engemann, J. J.; Fraimow, H. S.; and Abrutyn, E. (2004). Pathogens resistance to antimicrobial agents: Epidemiology, molecular mechanisms and clinical management. Infect. Dis. Clin. N. Am. 18: 467 – 511.

21. Hæggman, S., Löfdahl, S.; and Burman, L. G. (1997). An allelic variant of the chromosomal gene for class A beta-lactamase K2, specific for *Klebsiella pneumoniae*, is the ancestor of SHV-1. Antimicrob. Agents Chemother. 41(12):2705–2709.

22. Hæggman, S.; Löfdahl, S.; Paauw, A.; Verhoef, J. and Brisse, S. (2004). Diversity and evolution of the class Ac B – lactamase gene in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 48(7): 2400 – 2408.

23. Hennequin, C. and Forestier, C. (2007). Influence of capsule and extended spectrum beta- lactamases encoding plasmids upon *Klebsiella pneumoniae* adhesion. Research in Microbiology. 158(4):339-3347. (Abstract).