Al-Qadisiyah Journal of Pure Science

Volume 26 | Number 4 Article 16

8-15-2021

Molecular Detection Of Some Capsulargenes Of Klebsiella Pneumoniae Isolated From Clinical Samples

Amina A. Raheem
College of Science, University of Al-Qadisiyah, P.O.Box.1895, Iraq, aminaazoz7@gmail.com

Ghaidaa J. Mohammed
College of Science, University of Al-Qadisiyah, P.O.Box.1895, Iraq, ghaidaa.mohammed@qu.edu.iq

Recommended Citation
Raheem, Amina A. and Mohammed, Ghaidaa J. (2021) "Molecular Detection Of Some Capsulargenes Of Klebsiella Pneumoniae Isolated From Clinical Samples," *Al-Qadisiyah Journal of Pure Science*: Vol. 26: No. 4, Article 16.
DOI: 10.29350/qjps.2021.26.4.1355
Available at: https://qjps.researchcommons.org/home/vol26/iss4/16

Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: /10.29350/jops.
http://qu.edu.iq/journalsc/index.php/JOPS

Molecular Detection of Some Capsulargenes of Klebsiella pneumoniaeIsolated from Clinical Samples

Authors Names
a. Amina A. Raheem
b. Ghaidaa J. Mohammed

Article History
Received on:14 /6/2021
Revised on: 10/7/2021
Accepted on: 13/7/2021
Keywords:
Klebsiella pneumonia ,PCR, Capsular serotypes ,Clinical specimens

DOI: https://doi.org/10.29350/jops. 2021.26. 4.1355

ABSTRACT

Klebsiella pneumonia is an opportunistic pathogen that has been implicated as one of commonest cause of hospital and community acquired infections. The capsule of *Klebsiella pneumonia* is an important virulence factor, involved in pathogenic mechanisms. So, this study aimed to isolate *Klebsiella pneumonia* from different clinical specimens from patients in Al-Diwaniyah teaching hospital and determine some virulence factors (capsular genes)that used for serotyping of isolates. The study extended from August to November 2020.A total of 31 isolates from 80 different clinical specimens identified as *Klebsiella pneumonia* by traditional biochemical tests , Vitek system and 16SrRNA gene. The existence of three genes from7 tested capsular genes was detected by Polymerase chain reaction. The most common serotype were k2, k54, k57 where K2 detected in 4 (12.9 %), K54 in 12 (38.7 %),k57 in 4 (12.9).But, the other capsular polysaccaride genes k1, k3, k5,k20 not detected in all isolates of this study.

1. Introduction

Klebsiella pneumonia is an important opportunistic pathogen that causes a variety of infectious diseases in humans, including septicemia, liver abscesses, diarrhea, and pneumonia [9]. It is one of the known pathogens acquired in hospital and is associated with increased patient morbidity and mortality [2] .

K.pneumonia can express a variety of virulence factors including capsules, endotoxins, siderophores, iron scanning systems, and adhesins, which have been shown to play important roles in their etiology. The capsule is an important virulence factor, involved in at least two pathogenic mechanisms: (1) protecting bacteria from phagocytosis, and (2) inhibition of the host's immune response [12]. Some types of capsules (K), especially K1, K2, K54, K57 ,K20 and K5, are often associated with community-acquired invasive purulent liver abscess syndrome, septicemia, and pneumonia [11] . K2 and K5 are associated with community-acquired pneumonia [1]. K3 is generally associated with rhinoscleroma [11] .Capsules typing is currently the most used technique for typing *K. pneumonia*

a College of Science, University of Al-Qadisiyah, P.O.Box.1895, Iraq E-Mail: aminaazoz7@gmail.com
b College of Science, University of Al-Qadisiyah, P.O.Box.1895, Iraq E-Mail: ghaidaa.mohammed@qu.edu.iq

isolates and shows good reproduction in distinguishing clinical isolates [17]. Several PCRs targeting the *wzy* genes have been developed for capsule typing of *K. pneumonia* [4].The aim of this study was to detect the existence of some capsular serotypes genes in *K. pneumonia* isolated from different clinical samples (sputum, urine, burn, wound) because the important role of these genes in evade bacteria from phagocytosis.

2. Methodology

2.1. Bacterial isolation and biochemical identification

A total of(80) different clinical specimens including burns swabs, wounds swabs ,urine ,sputum were collected from patients in Al-Diwaniyah teaching hospital in Al-Diwaniyah city during a period from August to November 2020.The samples were placed in separate sterile plastic bags and then immediately transported to the laboratory in a cooler with ice packs (below 4°C) and processed within 2–4 h.

By sterile swab ,the sample was streaked onto MacConkey agar , followed by incubation at 37°C for 24 h. From MacConkey agar, three pink, mucoid colonies were picked up and subcultured onto nutrient agar at 37°C for 24 h, followed by identification by traditional biochemical tests , Vitek system (Biomerieux, France). Confirmed cultures were preserved in Brain-heart infusin broth containing 20% glycerol and stored at −20°C for further study.

2.2. Molecular Identification of *Klebsiella pneumonia* by 16S rRNA gene:

2.2.1. DNA extraction

A DNA extraction kit (Geneaid, Taiwan) was used to perform the process of extracting the DNA from fresh growth of *K. pneumonia* . The procedure was generated according to the manufacturer's protocol. The DNA was measured for quality and quantity using a NanoDrop(Nabi-Korea) .

2.2.2. Amplification of 16SrRNA gene by PCR

The primers (Forward primer: AGCGTCAGTCTTTGTCCAGG and Reverse primer; GATGACCAG CCACACTGGAA) used to amplify regions in the 16S rRNA gene have been designed by using program of Primer 3 Plus. The reaction of the PCR employed by using Mastermix (Bioneer, Korea) with at total volume (20 µl) of reaction mixture; including 5µl DNA template, 2µl (10pmol) for each primer, 11 µl of nuclease free water . The conditions of the thermocycler were at 95℃ for 2 min of initial denaturation, 35 cycles (denaturation at 95℃ for 30s, annealing at 58℃ for 30sec, and extention at 72℃ for 1 min), and final extension at 72℃ for 5 min. Using 1.5 %-agarose gel including 0.5µg/ml Red Safe dye , DNA bands were separated via electrophoresis and visualized via a Gel documentation system (Wisd-Korea).

2.3. Detection of Capsular serotype genes in *K. pneumonia*

All confirmed *K. pneumonia* isolates were grown overnight in nutrient broth at 37°C. Genomic DNA was extracted using a commercial universal DNA Extraction Kit (Geneaid- Taiwan) according to the manufacturer's instructions. Seven individual PCRs were performed to detect the presence of Capsule serotype genes(*k1,k2,k3,k5,k20,k54,k57*) in *K. pneumonia* isolates as in previous studies [1,20].Primer sequences, and amplicon sizes are shown in Table1.Amplified PCR products were analyzed by gel

electrophoresis on 1.5% agarose gels containing Safe Red(0.005% v/v) in 1 × TAE buffer, and imaged using Gel documentation system(Wisd-Korea).

Table (1): Primer sequences and reference of Capsule serotype genes used in this study

Target virulence genes	Primer Sequence(5′-3′)	Size (bp)	References
wzyK1 (magA)	F: GGTGCTCTTTACATCATTGC R: GCAATGGCCATTTGCGTTAG	1283	[19]
wzyK2	F: GACCCGATATTCATACTTGACAGAG R: CCTGAAGTAAAATCGTAAATAGATGGC	641	[19]
zxK5	F: TGGTAGTGATGCTCGCGA R: CCTGAACCCACCCCAATC	280	[19]
wzyK20	F: CGGTGCTACAGTGCATCATT R: GTTATACGATGCTCAGTCGC	741	[7]
wzxK54	F: CATTAGCTCAGTGGTTGGCT R: GCTTGACAAACACCATAGCAG	881	[7]
wzyK57	F: CTCAGGGCTAGAAGTGTCAT R: CACTAACCCAGAAAGTCGAG	1037	[13]
wzyK3	F: TAGGCAATTGACTTTAGGTG R: AGTGAATCAGCCTTCACCT	549	[8]

Table (2) : PCR Thermocycling conditions for all genes that used in this study

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	No. of cycles
magA(k1)	95C˚ 4 min	95C˚ 45 sec	50 °C 30 s	72C˚ 1 min	72C˚ 10 min	30 cycles
WzyK2	95C˚ 4 min	95C˚ 45 sec	52 °C 30 s	72C˚ 1 min	72C˚ 10 min	35 cycles
WzyK3	95C˚ 4 min	95C˚ 2 min	53 °C 30s	72C˚ 1 min	72C˚ 5 min	35 cycles
zxK5	95C˚ 4 min	95C˚ 30 sec	50 °C 30 s	72°C 1min	72°C 7min	30 cycles
wzyK20	95C˚ 4 min	95C˚ 30 s	50 °C 30 s	72C˚ 1 min	72C˚ 10 min	30 cycles
wzxK54	95C˚ 4 min	96C 30 s	50 °C 30 s	72C˚ 1 min	72C˚ 10 min	30 cycles

wzyK57	95C˚ 4 min	95C˚ 30 s	50 °C 30 s	72C˚ 1 min	72C˚ 10 min	30 cycles

3. Results & Discussion

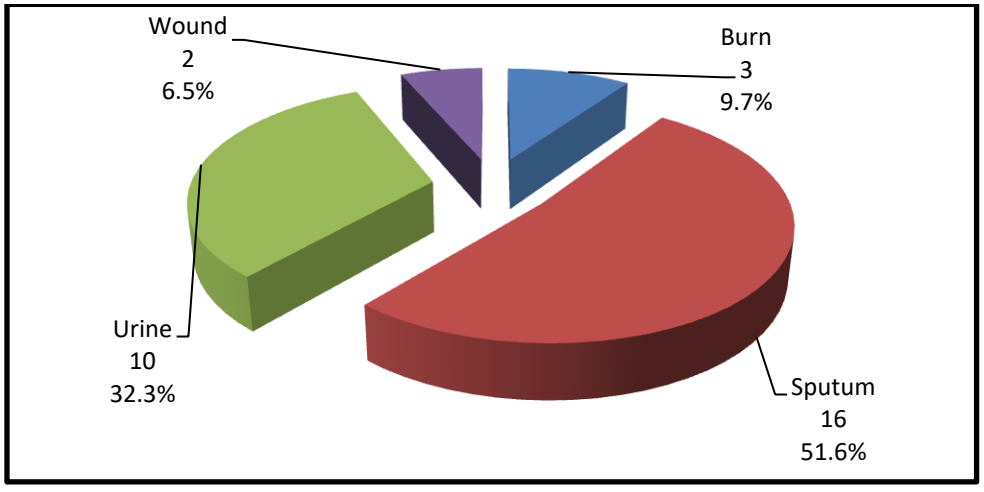
3.1. Prevalence of *K. pneumonia* in different clinical specimens

A total of 31 isolates were identified as *K. pneumonia* from (80) clinical specimens that collected from different clinical cases from Al-Diwaniyah teaching hospital in Al-Diwaniyah city/Iraq, by traditional biochemical tests and Vitek system; distributed as shown in Table (3) .In the study of Ssekatawa *et al*.[18], a variety of clinical specimens such as wound, burn, sputum and urine were used to study the prevalence of *K. pneumonia* in a hospital based study and they were able to isolate the micro-organisms from all these samples supporting our finding that *K. pneumonia* has the ability to cause a wide spectrum of clinical illness and can be isolated from a variety of clinical samples.

Table(3): The clinical specimens and rates (%) of each bacterial species from these specimens

Bacterial species	Burn		Sputum		Urine		Wound		Total	
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
E coli	3	42.9	3	9.4	10	40.0	5	31.3	21	26.3
Pseudomonas aeruginosa	0	0.0	0	0.0	0	0.0	7	43.8	7	8.8
Shigella spp.	0	0.0	8	25.0	0	0.0	0	0.0	8	10.0
Salmonella spp.	0	0.0	3	9.4	3	12.0	0	0.0	6	7.5
Klebsiella oxytoca	1	14.3	2	6.3	2	8.0	2	12.5	7	8.8
Klebsiella pneumonia	3	42.9	16	50.0	10	40.0	2	12.5	31	38.8
Total	**7**	**100.0**	**32**	**100.0**	**25**	**100.0**	**16**	**100.0**	**80**	**100.0**

The samples of *K. pneumonia* obtained in the current study were taken from four main clinical patterns: burn, urine ,wound and sputum as shown in figure (1). It should be mentioned that clinical samples yielded 31 isolates.



Figure(1). The clinical samples obtained in the current study from which *K. pneumonia* were isolated .

3.2. Molecular identification of *K. pneumoniae* by 16srRNA

It has been confirmed the identification of isolates by molecular detection of 16SrRNA gene as shown if figure (2). The 16SrRNA gene is used as a diagnostic tool for bacteria because it is found in all bacteria, even mutants. 16SrRNA is composed of highly conserved nucleotide sequences interspersed with variable regions of bacterial species and species. PCRs target protected areas of rRNA [3].

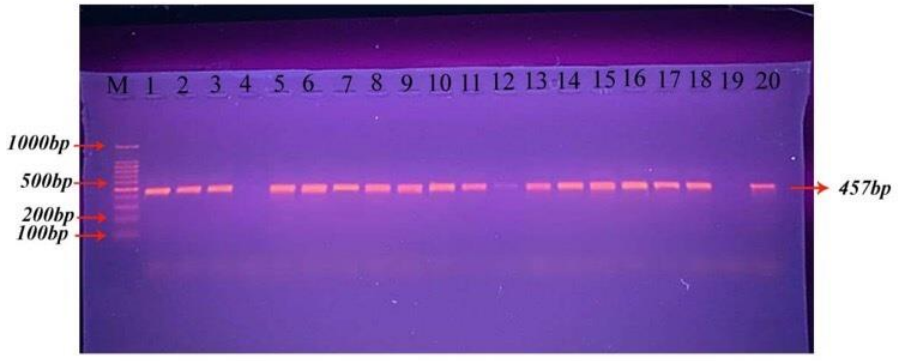


Figure (2) :Agarose gel electrophoresis image showed PCR product size at 457 bp for identification gene(16SrRNA) of *K. pneumonia* isolates. Marker ladder (100-1000bp), lane (1-20): Isolates numbers.

3.3. Detection of Capsular Serotype genes in clinical specimens

The serotype k1,k3 , k5 and k20 genes were not detected in *K. pneumonia* isolates . Regarding serotype K2 gene, it was identified in 12.9 % isolates of our study but was not detected in study of Hamam et *al*. [10] and Shakib *et al*. [14] . However, it was reported in 6.7 % in the study of Chen *et al*.[5] Serotype K54 detected in 37.8 % isolates of our study and 2.1 % in the isolates of study by Chen *et al*. [5] .Capsule serotype k57 in 25.8 % isolates of our study, while in the study of Ssekatawa *et al*. [18] there was no detection of K57 serotype gene. The K genes are capsular virulence factors involved in highly virulent *K. pneumonia* [5] .

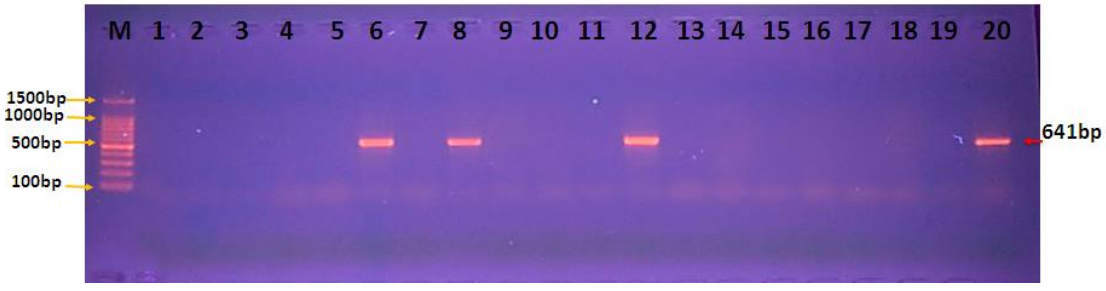


Figure (3): Image of Agarose gel electrophoresis showed PCR product of *k2* serotype gene in *K. pneumonia* Clinical isolates with product size 641bp. M:Marker ladder (1500-100bp), lane (1-20): Isolates number.

Figure (4): Image of Agarose gel electrophoresis showed PCR product of *k57* serotype gene in *K. pneumonia* Clinical isolates with product size 1037bp. M:Marker ladder (1500-100bp), lane (1-20): Isolates number.

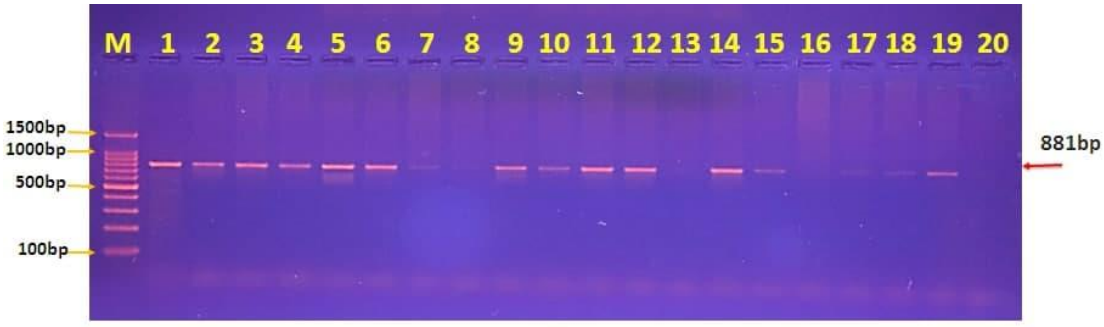


Figure (5): : Image of Agarose gel electrophoresis showed PCR product of *K54* serotype gene in *K. pneumonia* Clinical isolates with product size 881bp. M:Marker ladder (1500-100bp), lane (1-20): Isolates number.

K. pneumonia strains typically express both smooth lipopolysaccharide (LPS) with O antigen molecules and capsule polysaccharide (K antigen with different types as K1,K2,K3,K5, K20 ,K54, K57) confers resistance against the bactericidal activity of antimicrobial peptides, complement, and phagocytes. In addition, the capsule averts fulminate activation of the immune response[12] .

Several capsular (K) types, K2, K54, , K3, k57,are frequently linked to community-acquired invasive septicemia, pyogenic liver abscess syndrome and pneumonia[7,8,15]. Pathogen survival requires the acquisition of virulent factor such as capsule genes [6] and the acquired traits have been postulated to play an important part in the pathogenesis of *K. pneumonia* infections.

Conclusion: The high prevalence of virulent *K. pneumonia* among clinical isolates that get from the hospital (patient) as revealed by this study pose a great threat to healthcare. Our findings underline the epidemiological and public health risks and implications of this pathogen.

Conflict of Interest: The authors declare that they have no conflict of interest.

Acknowledgements

We extend our thanks to Biological Department /College of Science/University of Al-Qadisiyah and our thanks to all employees of the Lab units at Diwaniyah teaching hospital in Al-Diwaniyah city to facilitate the process of research.

References:

[1] Brisse, S.; Fevre, C.; Passet, V.; Issenhuthjeanjean, S.; Tournebize, R.; Diancourt, L., et al. (2009). Virulent clones of Klebsiella pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS ONE 4:e4982. 10.1371/journal.pone.0004982.

[2] Cabral ,A.B.; Melo Rde ,C.; Maciel, M.A.; Lopes, A.C.(2012). Multidrug resistance genes, including blaKPC and blaCTX-M-2, among Klebsiella pneumoniae isolated in Recife, Brazil . Rev Soc Bras Med Trop.Oct; 45(5):572-8.

[3] Chakravorty, S.; Helb, D.; Burday, M.; Connell, N. and Alland D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. Journal of Microbiological Methods, 69(2): 330–339.

[4] Cheng, L.; Cao, X. L.; Shen, H.; Zhang, Z. F.; Ning, M. Z.; Zhou, W. Q., et al. (2015). Investigations on the virulence, serotypes and genotyping of Klebsiella pneumoniae producing KPC-2. Chin. J. Clin. Lab. Sci. 33, 591–595. 10.13602 / j. cnki.jcls.2015.08.08

[5] Chen, Z.; Liu, M. and Cui, Y. et al. (2014).A novel PCR-based genotyping scheme for clinical Klebsiella pneumoniae. Future Microbiol. 9(1):21–32.

[6] da Silva, G.J. and Mendonça, N.J.V. (2012).Association between antimicrobial resistance and virulence in Escherichia coli. Virulence.;3(1):18–28.

[7] Fang, C.T.;Lai, S.Y.;Yi, W.C.;Hsueh, P.R.; Liu, K.L.and Chang, S.C. (2007). Klebsiella pneumoniae genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin Infect Dis 45:284–293. doi:10.1086/519262.

[8] Fevre, C.; Passet, V.;Deletoile, A.; Barbe, V.; Frangeul, L.; Almeida, A. S.,et al. (2011). PCR-based identification of Klebsiella pneumoniae subsp.rhinoscleromatis, the agent of rhinoscleroma. PLoS Negl. Trop. Dis. 5:e1052. doi: 10.1371/journal.pntd.0001052

[9] Guo, Y.;Wang, S.; Zhan, L.; Jin, Y.; Duan, J.; Hao, Z.; Lv, J.; Qi, X.; Chen, L.; Kreiswirth, B.N.; Wang, L.andYu, F.(2017). Microbiological and Clinical Characteristics of Hypermucoviscous Klebsiella pneumoniae Isolates Associated with Invasive Infections in China.Front Cell Infect Microbiol.; 7:24.

[10] Hamam ,S.; El Kholy,R.M.; Zaki,M.E.S.(2019). Study of Various Virulence Genes, Biofilm Formation and Extended-Spectrum β-lactamase Resistance in Klebsiella pneumoniae Isolated from Urinary Tract Infections. The Open Microbiology Journal 13(1):249-255 DOI:10.2174/1874285801913010249

[11] He, J. Y. (2012). Study of serotype and distribution on characterization of virulence genes of Klebsiella pheumonia [D]. Chongqing Medical University.

[12] Kang, Y.; Tian, P.; Tan ,T. (2015). Research advances in the virulence factors of Klebsiella pneumoniae-A review. Wei Sheng Wu Xue Bao,55(10):1245-52.

[13] Pan, Y. J.; Fang, H. C.; Yang, H. C.; Lin, T. L.; Hsieh, P. F.; Tsai, F. C. et al.(2008). Capsular polysaccharide synthesis regions in Klebsiella pneumoniae serotype K57 and a new capsular serotype. J. Clin. Microbiol. 46, 2231–2240.doi: 10.1128/JCM.01716-07

[14] Shakib ,P.; Kalani, M. T.; Rashid Ramazanzadeh, R. Amjad Ahmadi, A.; Rouhi,S.(2018). Molecular detection of virulence genes in Klebsiella Pneumoniae clinical isolates from Kurdistan Province. The Vietnamese Journal of Biomedicine. 5(8):2581 2589.DOI:10.15419/bmrat.v5i8.467

[15] Siri, G. P.; Sithebe, N. P.; and Ateba, C. N. (2011). Identification of Klebsiella species isolated from modimola dam (mafikeng) north west province – South Africa. Afr. J. Microbiol. Res. 5, 3958–3963. doi: 10.5897/AJMR11.690

[16] Sithebe, G.P. and Ateba,N.P.(2011). Identification of Klebsiella species isolated from Modimola dam (Mafikeng) North West Province South Africa. J Afr J Microbiol Res.; 5(23):3958–63.

[17] Siu L. K., Fung C. P., Chang F. Y., Lee N., Yeh K. M., Koh T. H., et al. (2011). Molecular typing and virulence analysis of serotype k1 Klebsiella pneumoniae strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. J. Clin. Microbiol. 49, 3761–3765. 10.1128/JCM.00977-11.

[18] Ssekatawa , K. ; Byarugaba, D.K. ; Nakavuma , J.L.; Kato ,C.D.; Ejobi ,F.; Tweyongyere , R.; Eddie, W.M. (2021). Prevalence of pathogenic Klebsiella pneumoniae based on PCR capsular typing harbouring carbapenemases encoding genes in Uganda tertiary hospitals. Antimicrob Resist Infect Control, 2021 Mar 18;10(1):57, doi: 10.1186/s13756-021-00923-w.

[19] Turton J. F., Baklan H., Siu L. K., Kaufmann M. E., Pitt T. L. (2008). Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in Klebsiella sp. and comparison of isolates within these serotypes. FEMS Microbiol. Lett. 284, 247–252. 10.1111/j.1574-6968.2008.01208.x

[20] Turton, J. F. (2010). PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. J. Med. Microbiol. 59, 541–547. 10.1099/jmm. 0.015198-0