Journal Of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

16S rRNA, *FimH1* and *magA* genes detection in *Klebsiella Pneumoniae* cultivated from CSF

Osamah Faisal Kokaz[1], Duaa Abd Alabass Muhammad[2]

1- Al- Muthanna University/ Medicine College, Samawah, Iraq.

2- Department of Pathological analyses, College of Science, University of Qadisiyah, AL-Diwaniyah, Iraq.

Corresponding author: ussama.faisal@mu.edu.iq

Abstract

Bacterial Meningitis is one of the main manifestations of *K. pneumoniae* infection in human in presence of multiple virulence factors. Sixty-four clinical sample from CSF were collected in one of the main hospitals of AL-Diwaniyah governorate of Iraq. 20 isolates were morphologically (culture media, biochemical tests) and genetically identified on the base of 16s rRNA using PCR instrument. Virulence factors that progress the pathogenicity of K. pneumoniae were confirmed by occurrence of FimH type 1 gene coding for fimbria in 10/20 (50%) located in 507 bp and biofilm forming megA gene in 15/20 (75%) amplified at 502 bp. The importance of these factors in dissemination of infection and resist to immune defense mechanisms.

Key words*: K. pneumoniae*, CSF, meningitis, PCR, FimH1 and megA.

Introduction

A family Enterobacteriaceae have many examples that causing numerous infections, *Klebsiella pneumoniae* (K. pneumoniae), as a member encapsulated, gram negative non-motile nosocomial pathogen (Martin & Bachman, 2018). These bacteria are known for causing numerous respiratory system disorders, invading the bloodstream, and causing serious infections. Furthermore, it is one of the most typically isolated bugs in

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

community-acquired infections (Podschun *et al*., 2001). Meningeal bacteria are life-threatening infection that continues to provide clinical challenges. *K. pneumoniae* is a pathogen responsible for <3% of community-acquired bacterial meningitis cases (Bartt, 2012). As an opportunistic pathogen*, K. pneumoniae* infects individuals whose immune systems have been enfeebled by an underlying disease such as diabetic, lung obstruction, or surgery; additionally, their clinical relevance in individuals whose immune systems are weakened by underlying disease is challenging to fix. (Kaul et al., 2007).

Three diverse forms can be illustrious in the medical scale of meningitis *K. pneumoniae*: (1) metastatic-meningitis, especially liver abscesses; (2) post-craniotomy meningitis caused by brain lesions or head injuries following neurosurgical procedures; and (3) spontaneous meningitis, which classically affects ageing patients or those underling immune system suppression (Lu *et al*., 1997). *K. pneumoniae* caused nearly 68% of severe meningitis in a subgroup of adult diabetic and fifty percent of severe meningitis in patients requiring critical upkeep (TANG et al., 1997), with great mortality percent located between 48.5 %- 66 % (Hsu *et al*., 2009; Huang *et al*., 2002).

Owing to its wide range of virulence factors, *K. pneumoniae* typically have a significant impact on the development of bloodstream infections, pyogenic liver abscesses, and pneumonia in human (Newire *et al*., 2013; Siu *et al*., 2011). These elements, which enable it to elude host immune responses, include the lipopolysaccharide, capsule, iron acquisition systems, adhesins, resistance to serum, and biofilm forming (Barreto *et al*., 2009).

The Enterobacteriaceae family's type 1 fimbria is the best understood; it is found in many species and is primarily made up of a number of structural subunits known as FimA. Clinical isolates of *K. pneumoniae* generate and amass types 1 and 3 fimbriae on their surface. FimH, an adhesin that gives mannose recognition capacity, is at the tip of the fimbria and interacts with FimA (Klemm & Schembri, 2000). It has been established that the hypermucoviscous phenotype, linked to protein MagA, confers hypervirulent *K.*

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

pneumonia (HvKP). Particularly in Asia, genes expressing MagA are strongly linked to hvKP and are regarded as virulence factors (Yu et al., 2006).

Conventional culture-based methods are still the major approach employed in clinical microbiology labs to identify bacterial isolates. These procedures can be costly, time-consuming, and need several biochemical tests—especially when dealing with picky organisms (Jin *et al*., 2011; Neil *et al*., 2010; Winstanley & Courvalin, 2011). Although automated identification devices have increased dependability recently like molecular technique ex: PCR, there have been reports of disparities throughout the different systems (Albuquerque et al., 2008; Kokaz, 2015).

According to their function in pathogenicity, the current investigation acknowledged the isolation and molecular detection of *K. pneumoniae* from cerebrospinal fluid (CSF) as well as the incidence of two significant virulence factors, adhesion FimH type1 and biofilm formation megA genes.

Materials and methods

1- Bacteria isolation

Under complete physician supervision, 64 CSF specimens were taken from patients hospitalized to Al-Diwaniyah Teaching Hospital between October and April of 2018. These samples were sent to the laboratory as soon as feasible in sterile transport media, where they were streaked on MacConkey agar (Macconkey, 1916). Overnight incubation at 37°C produced a pure colony of *K. pneumonia* that developed into a mucoid colony with a pink color when cultured on Eosin Methylene Blue (EMB. Tests for gram staining and biochemistry tests identified as *K. pneumoniae*. For future examinations, the isolated pathogen was kept at -4°C (MAHESH et al., 2017).

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

2- Molecular detection

The isolated pure colonies underwent DNA extraction in order to detect the 16s rRNA specific regions using the Polymerase Chain Reaction (PCR) instrument, following the manufacturing instructions provided by GENAID TAIWAN company. The primers were utilized according to the instructions provided in table 1, resulting in satisfactory amplification and visualization in gel electrophoresis. The presence of virulence factors, such as the adhesion FimH type1 and biofilm formation megA genes, was detected in certain previously confirmed isolates. This was achieved by using particular primers mentioned in table 1, following the instructions provided by the company. All primers in this work were designed by using 3plus primer software.

Figure 1: Primer that used to amplify the genes included in current study

Genes	Forword primers	Reverse primers	**bp**
16s rRNA	CTGTTCTGTCTGCACTGCG	CCCTTCACCCGGACTCATTT	515
FimH type 1	CCAGCCGTCCTGTATCTGAC	GATGATCGACTGCACGTTGC	507
megA	GACCGATGGTTGGGTTAGCT	GTTCACAACAGCTGCCTGAC	502

3- Statistical analysis

SPSS was used to perform the statistical work to weather finding some differences at 0.05 of probability as mentioned in (Al-Ukaelii, S. A., & Al-Shaeb, 1998).

Results

1- *K. pneumoniae* identification

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

In total, 64 samples were obtained from both sexes of individuals supposed to have meningitis, with the supervision of a physician. Notably no statistical differences were recorded considering the sex and ages. Out of the total, 20 cases (31.25%) were identified as *K. pneumoniae* based on their colony shape and results from biochemical tests, which included negative for oxidase, indole, motility, urease, and triple sugar iron tests (with both slant and butt appearing yellow).

2- Molecular characterization of *K. pneumonia*

A- Detection of 16s rRNA gene

The DNA extraction of *K. pneumonia* isolates revealed that all 20 out of 20 samples (100%) showed the presence of the universal 16S rRNA gene, which is the diagnostic gene for *K. pneumonia*. The size of the gene product was determined to be 515bp, confirming the presence of *K. pneumonia*. Below illustrated in figure 1.

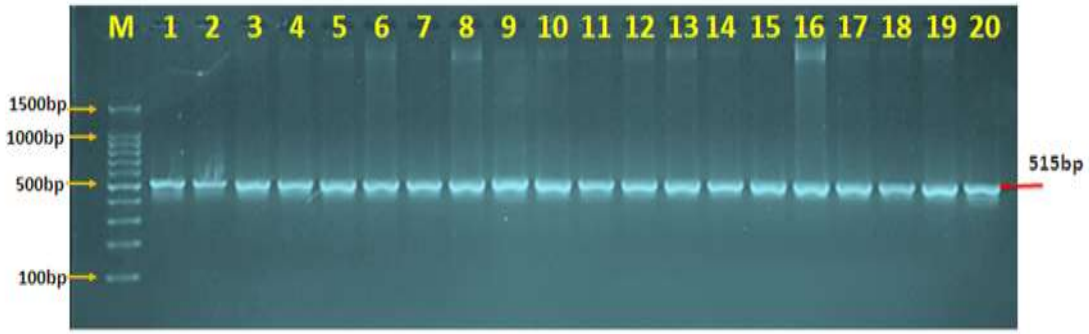

Figure (1): image of electrophoresis showed *K. pneumonia* PCR product analysis of 16S ribosomal RNA detection gene in. Where (1500-100bp) Marker ladder, positive K. pneumonia in Lane (1-20) 16S ribosomal RNA gene located 515bp PCR bands.

B- Molecular characterization of bacterial virulence factor genes

1- Adhesion

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

PCR was employed to determine the molecular identity of the fimH type I gene of *K pneumoniae* isolates. The investigation of 20 isolates showed in figure 2 that the gene desired a size 507 base pairs and was detected in 10/20 isolates (50.0%) of previously identified bacteria.

Figure (2): demonstrate the agarose gel electrophoresis of the PCR of the adhesion subunit of the fimH gene of *K. pneumonia* type I isolates where (1500-100bp) marker ladder, fimH gene Lane (1-20) recorded positive at 507bp of PCR.

B- Biofilms formation gene

The study utilized PCR to identify the molecular characteristics of biofilm mag A gene screened in *K. pneumonia* isolates. It was found that the 502bp gene was present in 15 out of 20 bacterial isolates, accounting for 75.0% of the total captured in figure 3.

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

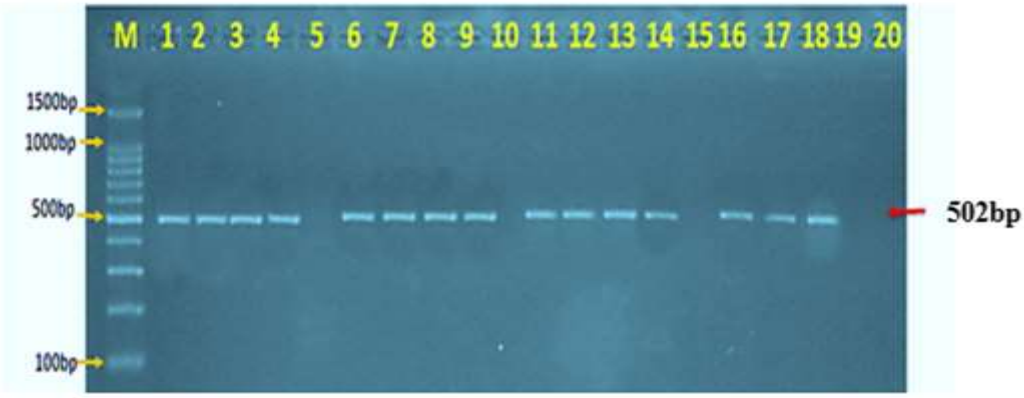

Figure (3): The agarose gel electrophoresis image showing the PCR product of the mag A gene associated with the *K. pneumonia* biofilm for a 502bp gene. M= lane (100-1500).

Discussions

Meningitis has much attention in research interest specially that caused by bacteria. *K. pneumonia* one the main causative agents that enrolled under investigation. Our study targeted isolation and molecular detection of this pathogen. Only 20 isolates were yields from 64 samples, that indicates the incidence of *K. pneumoniae* in CSF infections as early mentioned by Ku et al., (2017) whom isolated n= 33 *K. pneumoniae* from CSF and less frequency in study of Cruz-córdova et al.,(2014) about 10%. Other readings have been directed to assess the high efficacy of *Klebsiella* species identification (Haryani, 2018; Lau et al., 2007).

Virulence factors that aid in survive and dissemination of *K. pneumoniae* during the course of infection including adhesion FimH1. Half of total 20 express fimbriae type one gene implicated the distribution of this gene within *K. pneumoniae* strains. The main virulence factors associated with the pathogenesis of *K. pneumoniae* mare type 1 fimbriae, type 3 fimbriae, and capsule (Johnson et al., 2011; Johnson & Clegg, 2010).

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

Molecular methods have the accuracy to be number one in field of microbial detection as achieved in current study in case of FimH1 and MegA detection. 75% of CSF isolates have FimH1 which consistent with other study that found 98% of *K. pneumoniae* hold the same gene isolated from clinical settings (Cruz-córdova et al., 2014) and results of (Seifi et al., 2016) they found 93% of *K. pneumonia* presented ability of biofilm forming.

Reports from PCR analysis more accurate in the identification of *Klebsiella* species when likened with other ordinary identification examinations (Diancourt et al., 2005). Consequently, verification of *Klebsiella* identification consuming PCR inquiry is very valuable. Accurate ID of *Klebsiella* isolates is imperative for taxonomic and molecular categorization (Alves et al., 2006).

The existence of these virulent genes and depict of hvKP primary meningitis strains might assist in their ability to spreading in CSF space through compleat central nervous system barrier for illness, while the fewer virulent *K. pneumoniae* may be still be capable to cross the CSF space by disruption barrier of central nervous system (Ku et al., 2017).

Conclusion

K. pneumoniae are associated with meningitis and certainly isolated from CSF samples. The virulence factors that provide a substantial arm in pathogenicity term and both FimH type 1 and megA had crucial role in that process. Beside the genetic detection that no doubt confirms the presence of these genes in genomic material of *K. pneumoniae*.

References

Al-Ukaelii, S. A., & Al-Shaeb, S. M. (1998). *Statically Analysis by used SPSS program*. Al-Shoroq house for Publishers and advertisement Amaan, Jordan.

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

Albuquerque, P., Mendes, M. V, Santos, C. L., Moradas-ferreira, P., & Tavares, F. (2008). DNA signature-based approaches for bacterial detection and identification. *Science of the Total Environment, The*, *407*(12), 3641–3651. https://doi.org/10.1016/j.scitotenv.2008.10.054

Alves, M. S., Clayton, R., Christina, A., Castro, D. De, Riley, L. W., & Moreira, B. M. (2006). *Identification of Clinical Isolates of Indole-Positive and*. *44*(10), 3640–3646. https://doi.org/10.1128/JCM.00940-06

Barreto, S., Zambrano, M., & Araque, M. (2009). [Phenotypic variations of susceptibility in Klebsiella pneumoniae strains of nosocomial origin and their association with biofilm formation]. *Investigacion clinica*, *50*(2), 221–229.

Cruz-córdova, A., Esteban-kenel, V., Espinosa-mazariego, K., Ochoa, S. A., Espinosa, M., Garza, D., Rendón, E. F., López, O., & Xicohtencatl-cortes, J. (2014). *Hospital Infantil de México Pathogenic determinants of clinical Klebsiella pneumoniae strains*. *71*(1), 15–24.

Diancourt, L., Passet, V., Verhoef, J., Grimont, P. A. D., & Brisse, S. (2005). *Multilocus Sequence Typing of Klebsiella pneumoniae Nosocomial Isolates*. *43*(8), 4178–4182. https://doi.org/10.1128/JCM.43.8.4178

Haryani, Y. (2018). *Incidence of Klebsiella pneumonia in street foods sold in Malaysia and their characterization by antibiotic resistance , plasmid profiling , and RAPD – PCR Incidence of Klebsiella pneumonia in street foods sold in Malaysia and their characterization by an*. *July 2007*. https://doi.org/10.1016/j.foodcont.2006.04.009

Johnson, J. G., & Clegg, S. (2010). *Role of MrkJ , a Phosphodiesterase , in Type 3 Fimbrial Expression and Biofilm Formation in Klebsiella pneumoniae ᴐ*. *192*(15), 3944–3950. https://doi.org/10.1128/JB.00304-10

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

Johnson, J. G., Murphy, C. N., Sippy, J., Johnson, T. J., & Clegg, S. (2011). *Type 3 Fimbriae and Biofilm Formation Are Regulated by the Transcriptional Regulators MrkHI in Klebsiella pneumoniae ᴑ*. *193*(14), 3453–3460. https://doi.org/10.1128/JB.00286-11

Kaul, S., Brahmadathan, K., Jagannati, M., Sudarsanam, T., Pitchamuthu, K., Abraham, O., & John, G. (2007). One Year Trends in the Gram-Negative Bacterial Antibiotic Susceptibility Patterns in a Medical Intensive Care Unit in South India. *Indian Journal of Medical Microbiology*, *25*(3), 230–235. https://doi.org/10.1016/s0255-0857(21)02111-3

Klemm, P., & Schembri, M. A. (2000). IJ M Bacterial adhesins : function and structure. *International Journal of Medical Microbiology*, *290*(1), 27–35. https://doi.org/10.1016/S1438-4221(00)80102-2

Kokaz, O. F. (2015). Detection of mec A gene in Staphylococcus aureus from Osteomyelitis infections. *Al-Qadisiyah Medical Journal*, *11*(20), 184–189. https://doi.org/10.28922/qmj.2015.11.20.184-189

Ku, Y., Chuang, Y., Chen, C., Lee, M., & Yang, Y. (2017). Klebsiella pneumoniae Isolates from Meningitis : Epidemiology , Virulence and Antibiotic Resistance. *Scientific Reports*, 1–10. https://doi.org/10.1038/s41598-017-06878-6

Lau, H. Y., Clegg, S., & Moore, T. A. (2007). Identification of Klebsiella pneumoniae genes uniquely expressed in a strain virulent using a murine model of bacterial pneumonia. *Microbial Pathogenesis*, *42*(4), 148–155. https://doi.org/https://doi.org/10.1016/j.micpath.2007.01.001

Macconkey, A. (1916). LACTOSE-FEEMENTING BACTERIA IN FAECES . Lactose-Fermenting Bacteria in Faeces. *Briti Medical Journal Medical*, *1*, 188.

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

MAHESH, S., BASHA, P. A., & KAVITHA, B. (2017). ISOLATION AND CHARACTERIZATION OF BACTERIA ISOLATED FROM ISOLATION AND CHARACTERIZATION OF BACTERIA ISOLATED FROM MUNICIPAL SEWAGE WATER OF NANDYAL ,. *Asian Journal of Medical Biotechnology*, *19*(November), 772–777.

Martin, R. M., & Bachman, M. A. (2018). *Colonization , Infection , and the Accessory Genome of Klebsiella pneumoniae*. *8*(January), 1–15. https://doi.org/10.3389/fcimb.2018.00004

Newire, E. A., Ahmed, S. F., House, B., Valiente, E., & Pimentel, G. (2013). *Detection of new SHV-12 , SHV-5 and SHV-2a variants of extended spectrum Beta-lactamase in Klebsiella pneumoniae in Egypt*. 1–12.

Podschun, R., Pietsch, S., Höller, C., & Ullmann, U. (2001). Incidence of Klebsiella Species in Surface Waters and Their Expression of Virulence Factors. *Applied and Environmental Microbiology*, *67*(7), 3325–3327. https://doi.org/10.1128/AEM.67.7.3325-3327.2001

Seifi, K., Kazemian, H., Heidari, H., Rezagholizadeh, F., Shirvani, F., & Houri, H. (2016). *Evaluation of Biofilm Formation Among Klebsiella pneumonia e Isolates and Molecular Characterization by ERIC-PCR*. *9*(1), 2–7. https://doi.org/10.5812/jjm.30682

Siu, L. K., Fung, C., Chang, F., Lee, N., Yeh, K., Koh, T. H., Ip, M., & Icrobiol, J. C. L. I. N. M. (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*. *49*(11), 3761–3765. https://doi.org/10.1128/JCM.00977-11

ISSN: 0975-3583,0976-2833 VOL10, ISSUE 1, 2019

Yu, W., Ko, W., Cheng, K., Lee, H., Ke, D., Lee, C., Fung, C., & Chuang, Y. (2006). *Association between rmpA and magA Genes and Clinical Syndromes Caused by Klebsiella pneumoniae in Taiwan*. *2005*(April 2005), 27–29.