

Detection of Carbapenem-Resistant Genes and Specific Biofilm Association Genes in *K. Pneumoniae* Isolated from Medical Samples

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ABSTRACT

Background: *Klebsiella pneumoniae* is a ubiquitous encapsulated bacterial pathogen which cause various types of infections, this ability comes from the resistance gene and virulence factor genes.

Methodology: Fifty clinical isolates of *Klebsiella pneumoniae* was diagnosed and characterized according to their chemical and molecular characteristics, then genomic DNA was extracted from each bacterial isolate to detect carbapenem-resistant (*NDM-1*, and *OXA-1*), and biofilm association gene including (*fimH*, and *mrkD*) by amplification of these genes using specific primers.

Results: Results showed that *NDM-1* and *OXA-1* were found to be frequent in bacterial isolates in a percentage of 54% and 28% respectively. On the other hand, it was found that *fimH* is the most frequent in bacterial isolates (100%), while *mrkD* was found in a percentage of 86% of the total isolates.

Conclusion: High prevalence of biofilm association genes and presence of carbapenem-resistant in varied isolates of *K. pneumoniae* pose an important public health thing.

Keywords: *Klebsiella pneumoniae*, Antibiotic resistance genes, Virulence genes.

INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*), an associate to the Enterobacteriaceae family, Gram-negative bacteria, is a pathogen for human and animal⁽¹⁾. Increased mortality percent's are related with infection with carbapenemase creating *K. pneumoniae*⁽²⁾. Different carbapenemase genes, for instance: *K. pneumoniae* carbapenemase (KPC), VIM, OXA-48, NDM-1, and IMP have been recognized in *K. pneumoniae*^(3,4), several of which seem to be clone-specific and such clones are considered a pool for infection⁽⁵⁾.

The diseases of *K. pneumoniae* is interceded by some virulence factors that mediate to avoid hast innate immune reactions. The capsule, lipopolysaccharide, adhesins, iron acquisition systems, resistance to serum, and biofilm formation are considered as virulence factors responsible for *K. pneumoniae* pathogenicity⁽⁶⁻⁸⁾. In addition to Types 1 and type 3 fimbriae are formed and accumulated on the surface of experimental isolates of *K. pneumoniae*. All above factors are considered virulence factors⁽⁹⁾. Type 1 fimbria is one of the adhesion factors in numerous species of the Enterobacteriaceae family, contained the structural subunits called *FimA*. *FimH*, an adhesin that gives the capability for binding mannose (found at the tip of the fimbriae relating via *FimA*)⁽¹⁰⁾.

Type 3 fimbriae are responsible for the joining of erythrocytes treated with tannic acid, result in bacterial adherence to endothelial and bladder cell lines and have a role in biofilm creation on abiotic surfaces^(6,11).

Therefore, this experiment aimed to examine the carbapenem-resistant genes and biofilm association genes in KP isolated from clinical samples isolates from cases admitted to hospitals in Baghdad, Iraq.

MATERIALS AND METHOD

Clinical bacterial isolates:

Fifty isolates of *K. pneumoniae* collected from different clinical environments from September 2019 to December 2020, were cultured on MacConkey agar at 35-37 °C for 18-24 hours, then recognized by simple microbiological experiments excluding: catalase, MR-VP (Methyl red–Voges Proskauer), urea agar, indole, motility, blood agar, and MacConkey agar⁽¹²⁾. The isolates were stored at subzero (-20 °C) in a broth comprising glycerol for further examination.

Genomic DNA extraction:

Genomic DNA was extracted from each bacterial by using the protocol of Geneaid for genomic DNA extraction, then purity and concentrations of DNA solutions were determined by using Nano-drop spectrophotometer (Mettler, Germany).

Amplification of carbapenem-resistant genes and biofilm association genes. *NDM-1*, *OXA-1*, *fimH*, and *mrkD* were detected in *K. pneumoniae* isolates amplified by PCR amplification (Biomolecular system, Australia) using specific primers indicated in table (1).

The PCR situations were as follow: Initial denaturing at 94 °C for 5 min followed by 30 cycles, each cycle contained 1 min at 94 °C for denaturation, 30 second for annealing and 60 S for extension steps and finally one cycle for final extension at 72 °C for 10 min. After amplification, PCR yields were investigated on agarose gel to confirm the presence of the examined genes in the tested bacteria.

Table (1): The primer sequences of marked genes under experiment

Genes	Sequence {5' → 3'}	T _m (°C)	Reference
<i>NDM-1</i>	F: GGTTTGGCGATCTGGTTTTTC R: CGGAATGGCTCATCACGATC	52 °C	Candann and Aksöz ⁽¹³⁾
<i>OXA-1</i>	F: TTTTCTGTTGTTTGGGTTTT R: TTTCTTGGCTTTTATGCTTG	51.5 °C	Sugumar <i>et al.</i> ⁽¹⁴⁾
<i>FimH</i>	F: CGACCTCTCCACGCAGATTT R: CACGGTGCCTGAAAACTCG	62.5 °C	Catia <i>et al.</i> ⁽¹⁵⁾
<i>MrkD</i>	F: CGGTGATGCTGGACATGGT R: CCTCTAGCGAATAGTTGGTG	59 °C	Catia <i>et al.</i> ⁽¹⁵⁾

Ethical approval

This study was approved from The Research Ethics Committee, College of Science Baghdad University. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

The Statistical Analysis

System-SAS (2012) application was used to calculate the qualitative data, which were presented as frequency and percentage.

RESULTS AND DISCUSSION

Bacterial isolation and identification

Fifty bacterial isolates from different clinical samples were identified as *K. pneumoniae* according to their biochemical characteristic. Results showed the number and percentage of *K. pneumoniae* from clinical environments. These results exhibited that most isolates were collected from sputum 23 (46%), then wounds 10 (20%), urine 8 (16%), burns 3 (6%), ears 3 (6%), blood

2 (4%), and vagina 1 (2%). These results differ from those obtained by Hasan and Aburesha⁽¹⁶⁾ who found that the isolation percentage of *K. pneumoniae* for clinical specimens was 33.3% from blood, burn (24.24%), urine (21.21%), sputum (7.75%), wound (4.54%), and 3.03% from ear.

Molecular detection of carbapenem-resistant genes and biofilm association genes

Detection of NDM-1

NDM-1 gene was detected in *K. pneumoniae* isolates. Results of *NDM-1* amplification illustrated in figure (1) show that this gene was found in 27 (54%) off the total isolates with an amplicon size of 621 bp. The greatest important circulating NDM MBL gene in Enterobacteriaceae (*bla*_{NDM-1}) developed from *Acinetobacter baumannii*. This vision is pointed on the whole or different addition sequence IS*Aba*125 upstream of the *bla*_{NDM-1} genetic factor in mutually *bla*_{NDM}-harboring *A. baumannii* and Enterobacteriaceae⁽¹⁷⁾.

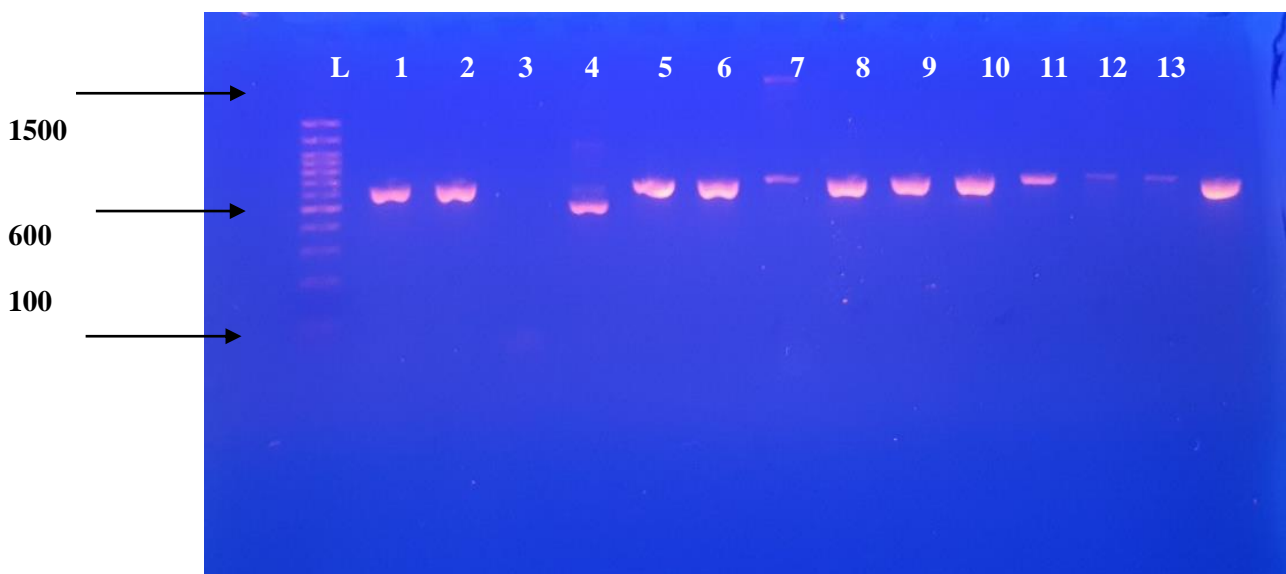


Figure (1): Amplification products of *K. pneumoniae* NDM-1 gene (621bp) after electrophoresis on 2% agarose gel for 50 min at 80 volt/ cm. Lane (L): DNA ladder marker; Lanes (1,2,5,6,8,9,10,14): positive for the gene. Lanes (3,4,7,11,12,13): negative for the gene

Detection of *OXA-1*

PCR screening was performed for identification of *OXA-1* using specific **primer** for identifying this known gene in *K. pneumoniae* isolates. Results of *OXA-1* amplification illustrated in figure (2) show that this gene was detected in 14 (28%) of the total isolates with an amplicon size of 441 bp. These findings are similar to **Sugumar et al.**⁽¹⁸⁾, who found that blaOXA-1 gene was noticed in 20.3% (12/59) of the studied *K. pneumoniae* separates.



Figure (2): Amplification products of *K. pneumoniae OXA-1* gene (441bp) after electrophoresis on 2% agarose gel for 50 min at 80 volt/ cm. Lane (L): DNA ladder marker; Lanes (7, 8, 12, 13): positive for the gene. Lanes (1,2,3,4,5,6,9,10,11,14): negative for the gene

Detection of *fimH*

Results of amplification illustrated in figure (3) show that fimbrial adhesion gene (*fimH*) was detected in 50 (100%) of *K. pneumoniae* isolates with an amplicon size of 512 bp. These results are in contract with **Stahlhut et al.**⁽¹⁹⁾ who mentioned that *K. pneumoniae fimH* gene was established in 90% of rinsing from numerous ecological and clinical bases. *FimH* is a paste subunit of type1 fimbriae voiced by diverse Enterobacteriaceae.

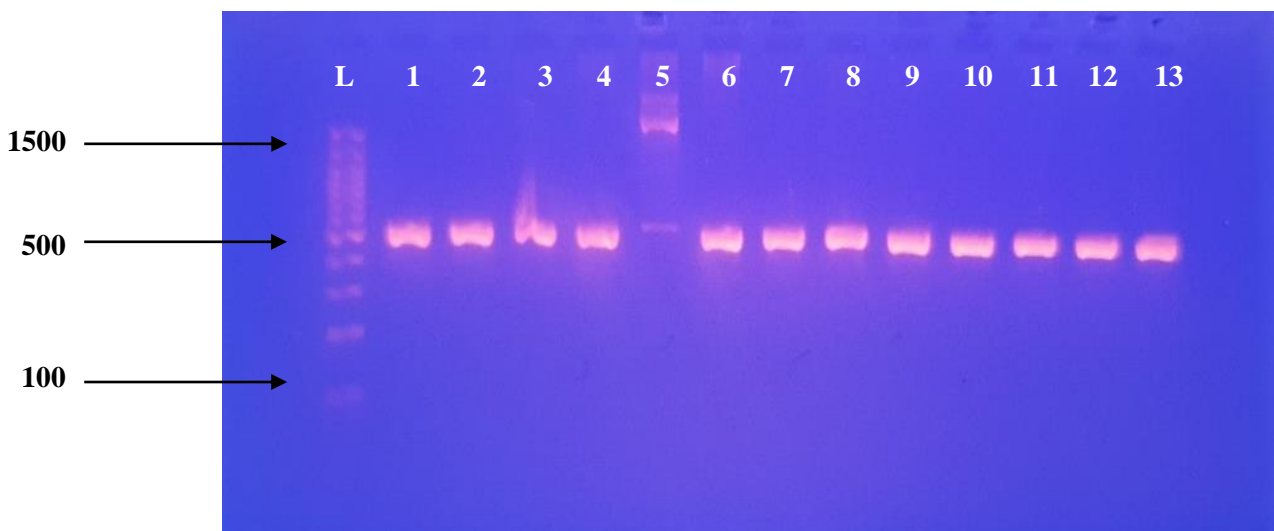


Figure (3): Amplification products of *K. pneumoniae fimH* gene (512bp) after electrophoresis on 2% agarose gel for 50 min at 80 volt/ cm. Lane (L): DNA ladder marker; Lanes (1,2,3,4,6,7,8,9,10,11,12,13): positive for the gene. Line (5): negative for the gene

Detection *mrkD*

Results of gel electrophoresis for the PCR product of *mrkD* illustrated in figure (4) show that fimbrial adhesion gene *mrkD* of 309 bp was detected in 43 (86%) isolates of *K. pneumoniae*. Type 3 fimbriae are owned by chaperon/usher type of fimbriae and are determined by the *mrkABCDF* gene mass, wherein *mrkD* encodes the chief basic constituent and *mrkD* translates the adhesion ability of fimbriae⁽²⁰⁾.

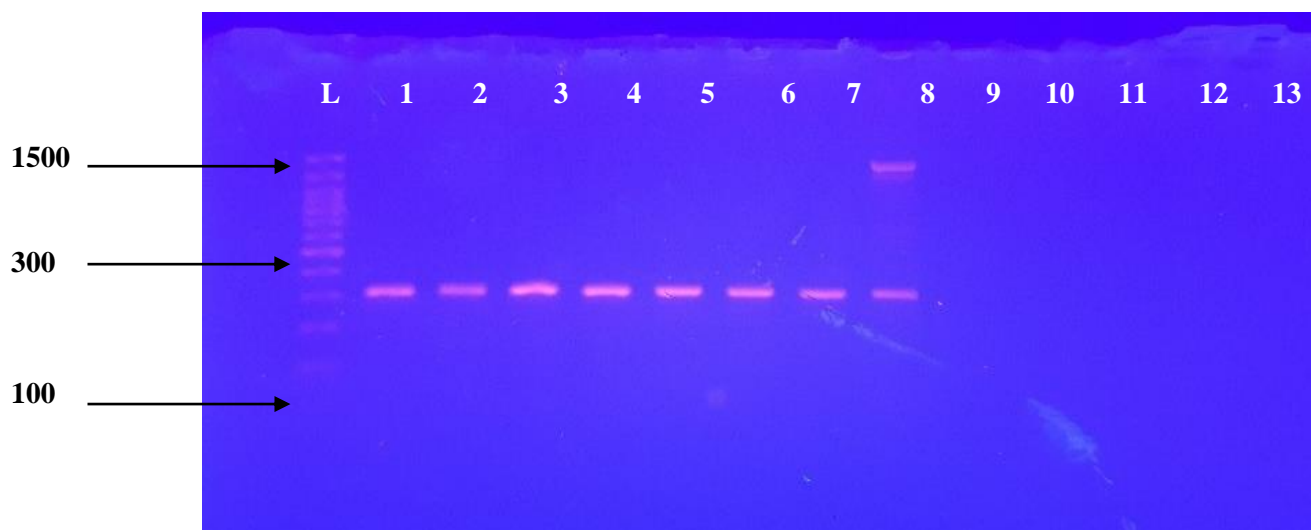


Figure (4): Amplification products of *K. pneumoniae mrkD* gene (309bp) after electrophoresis on 2% agarose gel for 50 min at 80 volt/ cm. Lane (L): DNA ladder marker; Lanes (1,2,3,4,5,6,7): positive for the gene. Lanes (9,10,11,12,13): negative for the gene. While lane (8) was nonspecific band.

Figure (5) shows the percentage and the occurrence frequency of the studied genes in *K. pneumoniae* isolates after PCR amplification of these genes and screening analysis. Appearance of *bla_{KPC}* and *bla_{NDM}* together in the same co-generating *K. pneumoniae* strains are considered the partial healing selections for clinical behavior. Understanding the variety and incidence of resistance and virulence genes of these separates is of unlimited meaning. These findings are similar to those obtained by **Xin *et al.***⁽²¹⁾ who found that the *bla_{KPC}* and *bla_{NDM}* co-creating *K. pneumoniae* strains have a high variety and incidence of resistance and virulence genetic factor, while **Ballén *et al.***⁽²²⁾ found that the *fimD*, *fimH*, *mrkC*, and *mrkD* genes were nearly universal between the strains (98.43%). This experiment may compromise hospitals vital evidence around the regulator of infections produced by *bla_{KPC}* and *bla_{NDM}* co-creating *Klebsiella pneumoniae*.

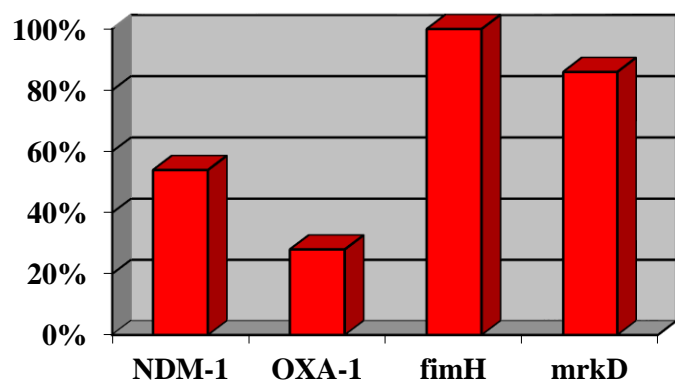


Figure (5): Percentage and prevalence of NDM-1, OXA-1, *fimH* and *mrkD* in *Klebsiella pneumoniae* isolates

CONCLUSION

Klebsiella pneumoniae has particular genes responsible for antibiotic resistance (*NDM-1*) and (*OXA-1*), and some virulence factor (*fimH*) and (*mrkD*). These isolates of *Klebsiella pneumoniae* are considered infectious isolates as they can cause infections.

Conflict of Interest: None

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