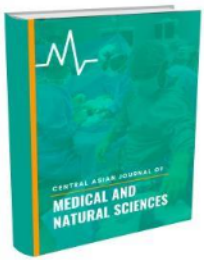

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Article

Molecular Investigation of Carbapenems KPC and NDM Genes Among Klebsiella Pneumoniae Strains Isolated from Various Clinical Samples in Al-Basrah Province, Iraq

Duha Hassan Abdul-Wahab[1], Saad Shakir Mahdi Al-Amara[2]*, Talib Abdulmaged Alboslemy[3]

1. M.Sc. Student, Department of Biology, College of Science, University of Basrah, Iraq
2. Department of Pathological analyses, College of Science, University of Basrah, Iraq

* Correspondence: saad.mahdi@uobasrah.edu.iq

3. Department of Biology, College of Science, University of Basrah, Iraq

Abstract: Carbapenem is the final defense against Gram-negative ESBLs producing antibiotics, with resistance to Enterobacteriaceae, particularly Carbapenem-resistant *Klebsiella pneumoniae* (CR-KP), posing a significant public health threat. The samples for the current investigation were from three hospitals in the Iraqi, region of Al-Basrah. The n = 26 *K. pneumoniae* isolates were previously collected by the master's study between October 2022 and December 2022. The *K. pneumoniae* isolates were reactivated and reidentified using a PCR technique using the diagnostic gene 16S rDNA. The isolates were then grown on MacConkey agar, *K.pneumoniae* chromogenic media and confirm identified by PCR technique by using specific primer for *K.pneumoniae* had a molecular weight of approximately 130 bp. The results showed that all n = 26 isolates are (100%) *K. pneumoniae*.Modified Hodge Test (MHT) was performed on all n=26 *K. pneumoniae* isolates.The results in the current study showed that out of n=26 *K.pneumoniae* isolates the 19(73%) isolates gave positive results for production of carbapenemase.While the 7(26.923%) isolates were showed negative results for produced carbapenemase. Carbapenemase genes was detected by using two specific primers *bla*$_{KPC}$ and *bla*$_{NDM}$ genes. The amplified genes' bands were characterized approximately at (480 bp) and (621) respectively, compared to the stander molecular DNA ladder at (2000 bp), results were shown the all n=26 *K. pneumoniae* isolates had gave negative results for the *bla*$_{KPC}$ gene. On the other hand, only 17(65.384%) *K.pneumoniae* isolates were gave positive results for detection of *bla*$_{NDM}$ gene. While 9(34.615%) *K.pneumoniae* isolates were gave negative results for detection the *bla*$_{NDM}$ gene.

Keywords: *Klebsiella pneumoniae*, carbapenemase, *bla*$_{KPC}$ and *bla*$_{NDM}$ genes

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1. Introduction

The final line of defense against Gram-negative extended spectrum ß-lactamases (ESBLs) producing antibiotics is carbapenem [1,2]. Resistance to carbapenems Enterobacteriaceae, in particular Carbapenem-resistant *Klebsiella pneumoniae* (CR-KP), are known to pose a significant threat to public health [3,4]. In 1996, *K. pneumoniae* carbapenemase was initially identified in the United States and subsequently dispersed throughout [5]. Carbapenemases are β-lactamases that hydrolyze penicillins, usually cephalosporins and carbapenems at different levels and monobactams except metallo-β-lactamases (MBL) [6]. The most commonly reported carbapenemases are class A (serine carbapenemases, such as *K. pneumoniae* carbapenemase (*KPC*), class B (MBLs, such as New Delhi MBL (*NDM*), Verona integron-encoded MBL (*VIM*), and imipenemase (*IMP*), and class D (*OXA* carbapenemase such as *bla*$_{OXA-48}$ [7, 8]. However, the increasing use of carbapenems has resulted in the

emergence of carbapenem-hydrolyzing β-lactamases (carbapenemases) as a common mechanism of resistance to carbapenem agents [9].

When treating serious infections caused by multidrug-resistant Gram-negative bacteria, such as *K. pneumoniae*, carbapenems are a dependable and efficient β-lactam antibiotic that are often used as a last resort [5]. Three pathways mediated carbapenem resistance in *K. pneumoniae*. First, there is the generation of carbapenemases; second, there is an outer membrane porin mutation coupled with an increase in AmpC β-lactamase synthesis; and third, there is the extended-spectrum drug efflux or porin mutation-induced β-lactamase (ESBL) synthesis. The most often mentioned resistance mechanism is carbapenemase synthesis [10]. This study aimed to detect *bla*$_{KPC}$ and *bla*$_{NDM}$ genes in carbapenem-resistant *K. pneumoniae* isolates from different clinical samples from the Al-Basrah province, Iraq.

2. Materials and Methods

Collection of Samples

The investigation were included n=26 *K. pneumoniae* isolates previously isolated during a master's study from three local hospitals in Al-Basrah, province, Iraq.The samples had been collected between October 2022 and December 2022 from urine, sputum, and wound swab samples from patients with UTIs, chest infections, and other illnesses. *K. pneumoniae* isolates were reactivated and re-identified according to [11,12] and kept in refrigerated until used in other tests.

Detection of Carbapenemase Producing Isolates

Klebsiella pneumoniae chromogenic medium

According to the manufacturer's instructions, the *K. pneumoniae* isolates were tested for the capacity to produce carbapenemase using the KPC chromogenic agar.

Modified Hodge Test (MHT)

According to CLSI, [13] the MHT test was performed on all n=26 *K. pneumoniae* isolates.

DNA Extraction

Using the Presto™ Mining DNA Bacteria, Geneaid, USA, kit procedure, for extracted genomic DNA from n=26 *K. pneumoniae* isolates.

Detection of 16S rDNA

The 16S rDNA amplification of the *K. pneumoniae* isolate DNA was carried out by PCR using a specific primer that had a length approximately 130 bp [14, 15]. standard molecular DNA ladder (2000 bp) was used compare the PCR results.

Detection of carbapenemase Genes

Two different specific primers that were utilized for amplified *bla*$_{KPC}$ and *bla*$_{NDM}$ genes by PCR with a particular primer that was approximately (480 bp) and (621 bp) in length respectively according to [16].

3. Results

The study examined n=26 K. pneumoniae isolates on MacConkey agar and K. pneumoniae chromogenic media, the results revealing mucoid pink, white, and purple colonies respectively Figure 1. PCR-based molecular diagnostics revealed a molecular weight of approximately 130 bp for all n=26 K. pneumoniae isolates, based on the 16S rDNA diagnostic gene as in Figure 2.

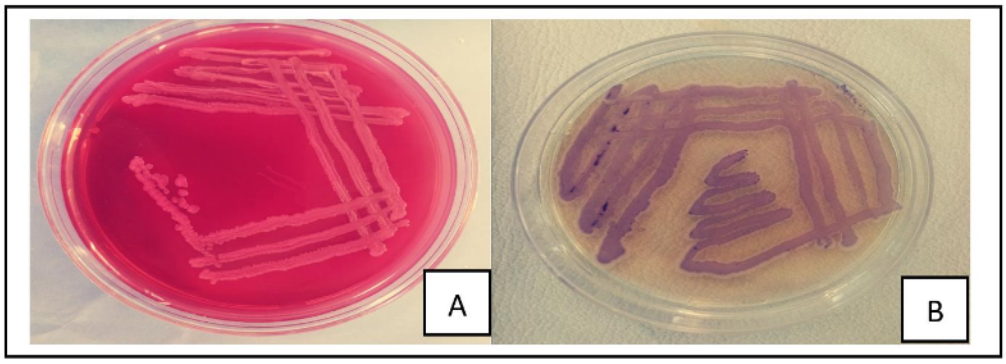

Figure 1. The *Klebsiella pneumonia* (A) on MacConkey agar, (B) *on K. pneumoniae chromogenic media*

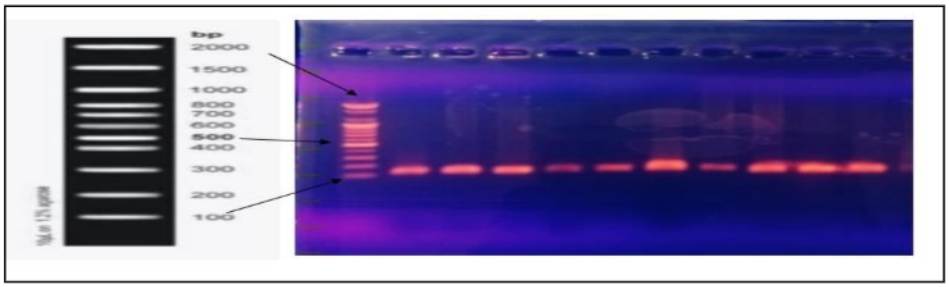

Figure 2. PCR Amplified Products of 16S rDNA. Lane 1:(2000 bp DNA ladder), Lane:(no. 2-11) 16S rDNA band of *K. pneumoniae* isolates using 1.5% agarose gel, 70V, 45min

The results in the current study showed that out of n=26 *K.pneumoniae* isolates the 19 (73%) isolates gave positive results for production of carbapenemase, while the 7 (26.923%) isolates were showed negative results for produced carbapenemase by using the modified hodge test (MHT). PCR was used to amplify the *bla*$_{KPC}$ and *bla*$_{NDM}$ genes. The amplified genes' bands were characterized approximately at (480 bp) and (621) respectively, compared to the stander molecular DNA ladder at (2000 bp), results were shown the all n=26 *K. pneumoniae* isolates had gave negative results for the *bla*$_{KPC}$ gene. On the other hand, the results of the current study showed that only 17(65.384%) *K.pneumoniae* isolates were gave positive results for detection of *bla*$_{NDM}$ gene. While 9(34.615%) *K.pneumoniae* isolates were gave negative results for detection the *bla*$_{NDM}$ gene by using PCR technique as in Figure 3.

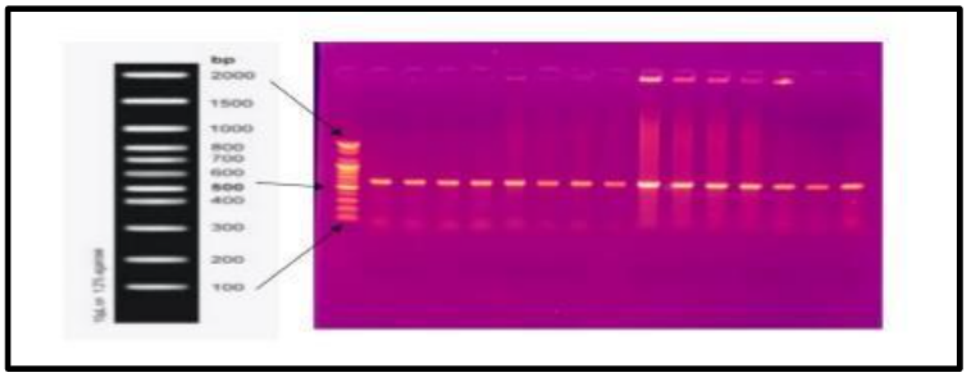

Figure 3. PCR Amplified Products of *bla*$_{NDM}$. Lane 1:(2000 bp DNA ladder), Lane:(no. 2-16) *bla*$_{NDM}$ band of *K. pneumoniae* isolates . using 1.5% agarose gel, 70V, 45min

4. Discussion

The n=26 *K. pneumoniae isolates* previously isolated during a master's study between October 2022 and December 2022 were reactivated by using brain-heart infusion broth and confirm identification through used MacConkey agar and *K. pneumoniae* chromogenic media. Lactose fermentation produced big, mucosal colonies on MacConkey agar, as lactose ferments, lactic acid is produced, while *K. pneumoniae* chromogenic media produced purple colonies [12]. Comparing this study to earlier research carried out in Erbil (95.45%) [17], Hilla hospitals (22%) [18], Iran (25%) [19], and Saudi Arabia hospitals (14.7%) [20], the prevalence rate of *K. pneumoniae* isolates is lower. The rate of *K. pneumoniae* isolation in China has increased and a high in 2020. The study found that the most prevalent organism in 13 (26%), sputum in 11 (22%), and wound swabs in 8 (16%) of the urine samples was *K. pneumoniae* infection [21]. Previous studies have demonstrated that 18 (36%) of the cases of *K. pneumonia* were isolated from sputum, 16 (32%) from blood, 9 (8%), from urine, and 7 (14%), from wound swabs. This is not the case anymore. *K. pneumoniae* is the second most common cause of UTIs associated with medical treatment [22].

On the other hand, in agreement with research conducted in Baghdad (20%), *K. pneumoniae* isolates have likewise demonstrated the greatest prevalence in urine sample frequency.[21] Egypt (50%), Duhok City (66.2%), and which accounts for 11(22%) of the isolates in the current investigation from sputum, is a frequent cause of hospital-acquired pneumonia. It is linked to ventilator-associated pneumoniae and a causal agent in severe infections such as surgical wound infections and septicemia. Hospital-acquired pneumonia is caused by the colonization of mucosal surfaces by *K. pneumoniae* [23]. Following the transmission of infections and detecting *K. pneumoniae* cases depend heavily on genotyping. Because genotypic characterization techniques may be adjusted to growth conditions, temperature, and environmental variables, they are more precise. Because it has a gene, 28 16S rDNA diagnosis is better than biochemical and phenotypic approaches [24,25]. For epidemiological purposes, the Modified Hodge test (MHT), which used for detects carbapenemases, might be a highly useful screening test for identifying those kinds of infections [26,27]. The CLSI suggests the MHT test as a method of identifying the synthesis of carbapenemase [26,28&29]. Out of n=26 *K. pneumoniae* isolates, 19(73%) isolates gave positive results for production of carbapenemase. While the 7(26.923%) isolates were showed negative results for produced carbapenemase. Study of [3] indicates that (52.17%) of *K. pneumoniae* isolates gave a positive result for MHT.Also the study of [31] the results were showed that out of 32 K. pneumoniae isolates, 16 (50%) showed positive results and 16 (50%) showed negative results for carbapenemase production. While the just 17% and 24% of isolates from study of [26] and [32] which produce positive results respectively. The results of MHT methods in current study, was confirm by diagnosis the *K. pneumoniae* isolates that produced carbapenemase depends on PCR, through used the specific primers for the *bla*$_{KPC}$ *and bla*$_{NDM}$ genes.The results showed all n=26 *K. pneumoniae* isolates gave the negative results for *bla*$_{KPC}$ genes. On other hand the results of the current study showed that only 17(65.384%) *K.pneumoniae* isolates were gave positive results for detection of *bla*$_{NDM}$ gene. While 9(34.615%) *K.pneumoniae* isolates were gave negative results for detection the *bla*$_{NDM}$ gene by using PCR technique. The study of [31] reported depending on the diagnostic the *bla*$_{NDM}$ gene showed that all (n=32) *K. pneumoniae* isolates revealed (100%) to *bla*$_{NDM}$ gene. The study of [33] refer to The two most significant enzymes among the well known varieties of carbapenemases are *bla*$_{KPC}$ *and bla*$_{NDM}$. As well, *bla*$_{NDM}$ gene expression was common in *K. pneumoniae* strains resistant to carbapenem, whereas *bla*$_{KPC}$ gene expression was absent from all *K. pneumoniae* isolates.

5. Conclusion

Klebsiella pneumoniae that is resistant to carbapenems is a significant hazard to public health. In local laboratories and hospitals, the Modified Hodge Test (MHT) is helpful in determining the production of carbapenemase. To identify carbapenem-resistant *Klebsiella*

pneumoniae and its possible horizontal gene transfer, correlations between antibiotic resistance profiles and carbapenem-resistant gene PCR patterns are essential to detect the carbapenems resistance isolates.

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