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Detection Sequencing NDM and blaOXA Genes in Metallo-β-Lactamase Producing Klebsiella Pneumoniae

Rahma Ali Alhadi Hussain[1,*], Ashwak Jasim Kzar[2] and Alaa Salim Hamza[2]
[1]Department of Medical Laboratory Technology, College of Health and Medical technique, Middle Technical University, Iraq.
[2]Department of Anesthesia Technique, Institute of Medical Technical /Baghdad, Middle Technical University, Baghdad, Iraq.

Corresponding author: Rahma Ali Alhadi Hussain (e-mail: rahma.ali.9191@gmail.com).

Abstract The prevalence of Carbapenem-resistance in Enterobacteriaceae is rising worldwide and poses a treatment challenge for healthcare facilities. This study aims to detect a sequence of New Delhi metallo-β-lactamase (NDM) and bla OXA-48 gene in metalo-beta-lactamase-producing Klebsiella pneumoniae isolated from a few hospitals in the city of Baghdad. 74 Klebsiella pneumoniae were isolated from 320 clinical samples that were routinely submitted to the diagnostic laboratory of the chosen hospital. These samples included sputum, tissue swabs, folly tips, urine, ear swabs, bloodstream, pus, wound swabs, endotracheal tubes, and body fluids. In this study, 74 isolates were examined for expression of carbapenemase by phenotyping method. The modified Hodge Test (MHT) is utilized for phenotypic detection. MHT was positive for 24 carbapenem-resistant Klebsiella pneumoniae. Genotyping was accomplished by using polymerase chain reaction (PCR) to amplify target genes by employing particular primers. The NDM gene was detected in 19/24 isolates, and the blaOXA-48 gene was detected in 5/24 isolates. The presence of bla NDM and the dangerously high frequency of MBL in the Klebsiella pneumoniae strain are both concerning, and it has been demonstrated to be challenging to treat and inhibit infection.

Key Words NDM, Metallo-β-lactamase, blaOXA-48, Klebsiella pneumoniae

1. Introduction

More and more reports of carbapenem-resistant Enterobacteriaceae are appearing worldwide, a significant problem for healthcare systems [1], [2]. MBLs can produce a wide variety of beta-lactamase activities, potentially negatively impacting many beta-lactam drugs, including carbapenems [3]. The plasmids that code for ESBLs can be transferred from one species to another, which presents a significant health risk, especially to those who have gotten to hospitals [4], [5]. Klebsiella pneumoniae is characterized by a polysaccharide capsule and is a rod-shaped, gram-negative, lactose-fermenting bacillus [6]. Potentially result in several infections, most frequently pneumonia, soft tissue, wound, and urinary tract infections [7]. Two main processes support K. pneumoniae resistance to carbapenems: i) synthesis of extended-spectrum-lactamases (also known as cephalosporins or ESBL) linked to porin depletion and ii) synthesis of carbapenem-hydrolyzing metallo-lactamases like class A carbapenemases, class B metals-beta-lactamase (VIM, NDM- or IMP-type) or class D carbapenemase OXA-48 [1], [2], [8], [9].

In 2009, NDM was initially discovered from a strain of Klebsiella pneumoniae that was highly contagious and resistant to many drugs [10]. Recent studies have led to the discovery of an NDM enzyme capable of conferring resistance to all β-lactams, in particular carbapenems [11]. In 2004, the OXA-48 carbapenemase was originally reported as being found in Turkish isolates [8]. OXA-48-producing bacteria commonly create several resistance mechanisms, some of which lead to multidrug resistance. These mechanisms include extended-spectrum-β-lactamases (ESBLs) and other resistance determinants.

Depending on the population's age and the condition's sickness profile, The percentage of deaths attributed to infections brought on by Klebsiella pneumoniae resistant to carbapenems could reach as high as 75% of the whole population [12]. According to the recommendations of the CLSI, carbapenemase-producing strains can be detected by utilizing several phenotypical approaches, such as the Modified Hodge Test (MHT) [13]. Following the recommendations provided by CLSI, The MHT was applied to identify carbapenemases-producing strains [10], [14]. The current work used NDM and bla OXA gene sequencing to discover metallo-β-lactamase-

Primer	Sequence (5'-3')		Anneal ing Temp. ($^{\circ}C$)	Product size, bp	Reference
OXA-48	F	CCA AGCATT TTTACC CGCATC KACC	55	338	16
	R	GYTTGACCATACGCTGRCTGCG			
NDM	F	GGTTTGGCGATCTGGTTTTC	52	621	9
	R	CGGAATGGCTCATCACGATC			

Table 1: Primers used for the Detection of Klebsiella pneumoniae

producing Klebsiella pneumonia.

2. Methods

Collection and Identification of Bacterial Strains

Three hundred twenty samples of sputum, tissue swab, folly tip, urine, ear swab, bloodstream, pus, wound swab, endotracheal tube, and body fluid were analyzed. A total of 74 K. pneumoniae were isolated from those samples by males and females from December 2022 to March 2023 from Iraqi patients in a few chosen hospitals in Baghdad city. The biochemical test, VITEK 2 compact system, was used to identify the isolated K. pneumoniae.

Carbapenemase Assay

Carbapenemase production investigated by a modified Hodge test was carried out [15]. After bringing a suspension of E. coli (sensitive strain) to a turbidity of 0.5 Mcfarland standard, it was injected equally on a Muller Hinton agar plate. After that, A disc containing (10) μg of meropenem was positioned precisely in the middle of the plate. The strains being tested were applied from the edge of the disc to the edge of the plate. The plate is allowed to incubate for 16-18 hours at $35^{\circ}C$.

PCR Amplification and Sequencing

DNA extract from the bacterial isolates, the ABIOpureTM kit was utilized. A polymerase chain reaction (PCR) analysis using specific primers was carried out in order to look for carbapenemase genes in Klebsiella pneumoniae: blaNDM and blaOXA-48 (Table 1). The reaction was carried out using 20μl volumes containing 10μl GoTaq Green Master Mix (2X), 1μl for each primer (10pmol), 6μl nuclease-free water, and 2μl of template DNA. With the use of PCR Express, PCR cycling was carried out(Thermal et al., USA) utilizing the temperature program that is as follows: denatured at 94 degrees Celsius for four minutes, then subjected to thirty cycles of denaturation at 94 degrees Celsius for thirty seconds, annealing at 55, 58, 60, 63, or 65 degrees Celsius for thirty seconds, and extension at 72 degrees Celsius for thirty seconds. After a final extension incubation of seven minutes at 72 degrees Celsius, the reactions were stopped by an incubation lasting ten minutes at four degrees Celsius. Macrogen Corporation - Korea utilized an automated DNA sequencer known as the ABI3730XL to perform Sanger sequencing on the PCR results.

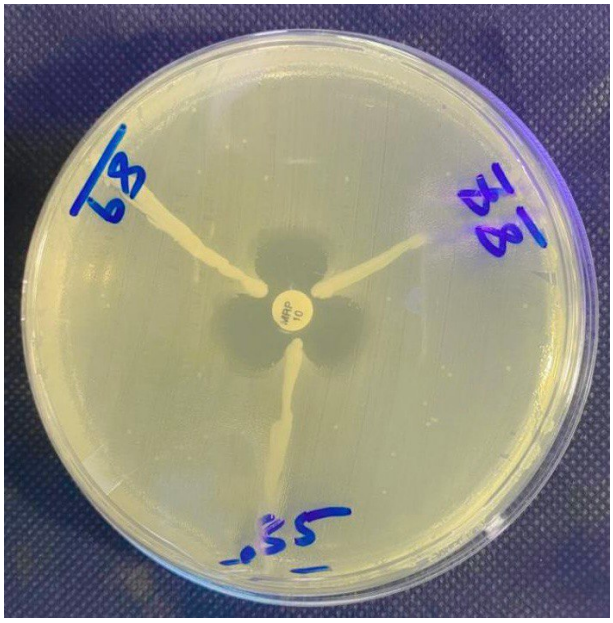

Figure 1: Inhibition test for detection of MBL producer by MH test.

3. Result

Bacterial Isolation

After laboratory identification using conventional morphological and biochemical tests as well as confirmation by VITECK, there were 74 isolates of Klebsiella pneumoniae. distributed urine ($n = 17$), foly tip ($n = 5$), sputum ($n = 18$), blood ($n = 4$), wound swab ($n = 6$), body fluid ($n = 12$), pus ($n = 5$), ETT ($n = 2$), tissue biopsy ($n = 3$), ear swab ($n = 2$). The rate among the strain of K. pneumoniae isolated from sputum and urine was higher than other isolates, while the lowest rate was observed among the strain isolated from ear swabs and end ETT. Isolates were found in individuals aged 1 to 60 years old, with 43.2% found in males and 56.7% in females.

Phenotyping Detection of Carbapenemase Producer

The 74 bacterial isolates were placed through screening tests to determine their carbapenemase production. The findings indicate that the MHT was positive for the presence of 24 Klebsiella pneumoniae strains resistant to carbapenem. A positive result for the MH test is characterized by a deformed inhibition zone, which appears like a clover leaf Figure 1.

PCR amplification

The ABIOpureTM kit made the DNA extraction for 24 carbapenemase-producing K. pneumoniae. The purity and concentration of DNA ranged from 21 -29 ng/μl. PCR analysis, which was then followed by sequencing, shows the presence of the NDM gene in in 19/24 isolated Figure 2 and The blaOXA-48 gene in 5/24 isolated Figure 3.

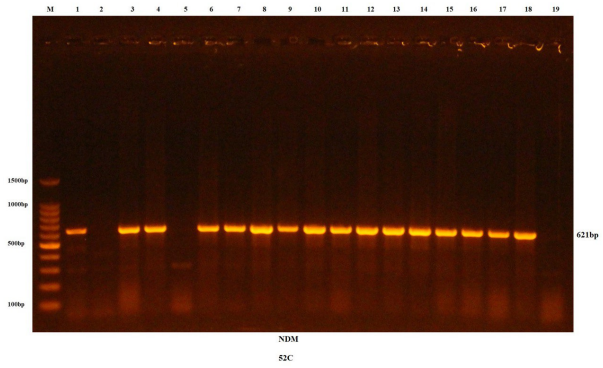

Figure 2: Results of the amplification of the NDM gene of Klebsiella pneumoniae

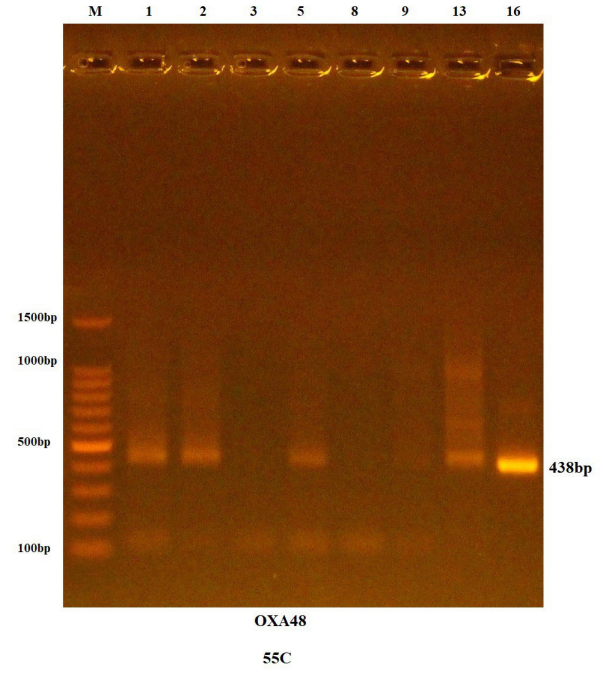

Figure 3: Results of the amplification of the OXA48 gene of Klebsiella pneumoniae

Sequencing of Metallo-β-Lactamase Genes

The sequencing of 5 virulence genes (NDM, blaOXA-48) from K. pneumoniae showed genetic variation in nucleotide sequence compared to world isolates, As shown in the phylogenetic tree.

Phylogenetic Tree of Sequencing Target Genes

OXA 48 virulence genes are divided into two groups: sequencing of group one (1-OXA48-F.ab1, 2-OXA48-F.ab1, 5-OXA48-F.ab1, 13-OXA48-F.ab1)are similarity to the world isolates genes (CP113224, CP113198, CP083935). There is a difference in sequencing between group two (16-OXA 48-F.ab1) and world isolate genes (CP1100598, CP114756), as shown in (Figure 4). NDM virulence genes

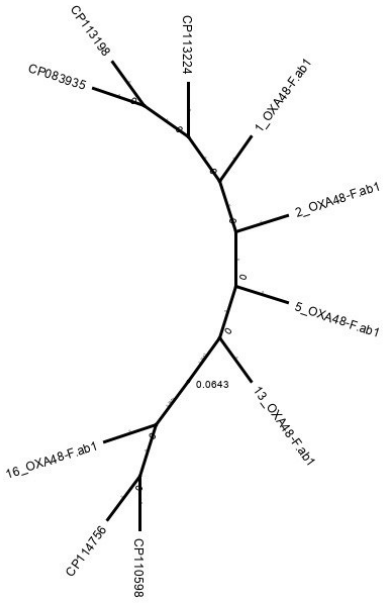

Figure 4: Phylogenetic Tree of oxa-48 Gene in Locally Clinical Isolates Klebsiella pneumoniae (1,2,5,13,16) Comparison with World Klebsiella pneumoniae in NCBI (CP110598, CP114756, CP113198, CP083935).

are divided into two groups: sequencing of group one (1-NDM-F.ab1, 13-NDM-F.ab1, 14-NDM-F.ab1, 15-NDM-F.ab1) is similar to the world isolates genes (ON572245, CP102689, CP101837). There is a difference in sequencing between group two (16-NDM-F.ab1) and world isolate genes (CP113224, CP113198, CP083935) (Figure 5).

Documentation of Local Bacterial Genes in NCBI

Documentation genes from isolates of Iraqi virulence K.pneumoniae were carried out as a new Iraqi strain. Two selected isolates of virulence genes, K.pneumoniae, have a specific sequence of nucleotides with genetic variation to the world strain. The results showed the accepted sequence of two gene isolates in NCBI, Klebsiella pneumoniae Iraq 53 blaOXA gene(LC765340) and Klebsiella pneumoniae Iraq 54 NDM gene(LC765339).

4. Discussion

Klebsiella pneumoiae has emerged as a major infectious agent responsible for 20% of all infections in hospitals globally [16]. Antibiotics known as carbapenems are the most effective medications for treating illnesses caused by bacteria because they exhibit antibacterial activity against various bacteria [17]. Greater death rates are associated with infections that are caused by K. pneumoniae strains that are resistant to carbapenems, length of hospitalization, and expense of therapy. Additionally, the number of strains of K. pneumoniae resistant to carbapenem is increasing [18].

In our result, $n = 24(32.4\%)$ of K. pneumoniae strains show phenotyping positive results for MBL by using MHT.

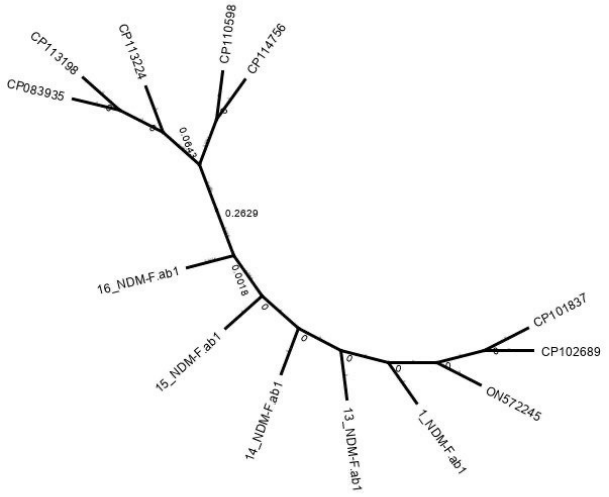

Figure 5: Phylogenetic Tree of NDM Gene in Locally Clinical Isolates Klebsiella pneumoniae (1, 13, 14, 15, 16) Comparison with World Klebsiella pneumoniae in NCBI (ON572245, CP102689, CP101837, CP114756, CP1105998, CP113224, CP113198, CP083935).

According to the findings of a recent investigation, the MHT is not very effective in identifying metallo-lactamase-generating Enterobacteriaceae [19]. In another research that was carried out in Greece, it was discovered that 50% of K. pneumoniae blood isolates contained metallo-beta-lactamases [20]. Various techniques or clinical samples could be to blame for the varied prevalence of MBL generation among K. pneumonia strains in multiple investigations. In addition, there are variations in the phenotypic and genotypic traits and the economic standing of bacterial isolates found in different regions of the world.

The NDM gene, which is only found in members of the Enterobacteriaceae family, sets ESBL distinct from other beta-lactamases. It is responsible for the bacteria's ability to withstand treatment with any beta-lactam antibiotic [21]. K. pneumoniae that tested positive for the New Delhi metallo-beta-lactamase gene in 2010 was found to have been imported from India and had spread to the UK. Five years after its discovery in Sweden in 2008, NDM was discovered in Brazil [22]. In another study in Kashan, Iran, 11.1% of K. pneumoniae isolates carried blaNDM gene discovery [23]. The result of our PCR demonstrated that a high positive rate of carbapenemase-producing K. pneumoniae had the NDM gene 79.1%. This data showed that the NDM spread quickly, and alternative therapies are required for infections brought on by these isolates.

For cephalosporins and monobactams, locating Enterobacteriaceae that produce OXA-48 can be very important. On the other hand, this feature can make it difficult to find them. In the fight against K. pneumoniae, The OXA-48-type carbapenemase is an essential immune system component [24]. PCR analysis in this study showed the presence of the blaOXA-48 gene in 5(20.8%) K. pneumoniae isolates. Therefore We can expect the spread of blaOXA-48in several countries in the Middle East.

The phylogenetic tree revealed that locally sequenced genes are similar to global strains in NCBI of the same bacteria, and some sequenced genes contain genetic variation there we chose and accepted as Iraqi strains in NCBI.

5. Conclusion

The majority of K. pneumoniae isolates observed in this investigation exhibited resistance to MBL formation, which is concerning, particularly among patients who were hospitalized. Whereas the spread of the NDM and production of bla OXA-48 may be more widespread in Iraq and can be expected in any country in the Middle East.

Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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