Molecular characterization of *Klebsiella pneumoniae* associated with Thalassemia in Thi-Qar Governorate

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Abstract: *Klebsiella pneumoniae* is a gram-negative, aerobic, non-motile bacilli and is a common cause of a wide range of infections in humans, *Klebsiella* spp. a major causative bacteria in Thalassemia patients. The present study aimed to shed light on the supposed role of bacterial infection in Thalassemia patients during the period from August 2022 to December 2023 in Genetic Blood Diseases/Thi-Qar Governorate. Forty blood samples were collected from all patients and subjected to Conventional Polymerase Chain Reaction assay by using the Universal gene. Nine PCR products were selected and subjected to partial DNA sequencing for the 16S rRNA gene to follow up their possible relationship between them and what was recorded globally in Gene bank. The results revealed that 9/40 (25%) isolates were *K. pneumoniae*. PCR product of 16S rRNA was recorded globally in Gene bank under the official accession numbers of OQ928953.1, OQ929666.1, OQ928950.1, OQ929250.1, OQ927231.1, OQ928942.1, OQ927249.1, OQ927229.1 and OQ927253.1.

The phylogenetic tree that was constructed by MEGA-10 software showed that there were different molecular relationships among the local *K. pneumonia* isolates with analogous ones around the world.

Keywords: Thalassemia, *Klebsiella pneumoniae*, Gene sequencing, phylogenetic tree.

I. INTRODUCTION

Thalassemia is an autosomal recessive common genetic disorder throughout the world [1]. Thalassemia is an inherited impairment of hemoglobin production, in which there is partial or complete failure of synthesis of a specific type of globin chain, the defect may affect the α, β, γ and δ chain or may affect some combination for this type, and named according to the type of chain defect [2]. It is a major health problem all over the world where the value of hemoglobin (the main component of the red blood cells and oxygen transporter) is below normal [3,4]. Clinically, thalassemia can be divided into three types, namely thalassemia major, thalassemia minor, and thalassemia intermediate[5,6]. One of the most common causes of death in thalassemia patients is bacterial infection. Several studies have indicated that infection is common in thalassemia patients, and that more than 10% of these infections were severe, that the main causes of infection were directly linked to blood transfusions[7]. Microorganisms such *Yersinia enterocolitica*, *Klebsiella* spp., *Escherichia coli*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* have been reported in the state of iron overload. A previous study revealed that splenectomy predisposed thalassemia and sickle cell disease patients to severe infections mostly by Gram-negative microorganisms [8]. Polymerase chain reaction (PCR) is considered the most well developed molecular technique for the detection of various diseases [9]. 16S rRNA gene sequencing provides confident results. This gene is consider one of the major criteria in the classification because of their regions which were highly stable and unable to change over time also they contain areas of highly variable among types of bacteria so that they provide a specific sequence for each type. This appears why this gene plays an important role in the diagnosis[10].

II. PATIENTS & METHOD

Samples Collection: A total of 40 blood samples were collected from patients of both gender of different ages who suffered from Thalassemia. All patients have consulted by the Genetic Blood Disease Center in Al-Nasiriya City, Southern Iraq from August 2022 to December 2023.

Molecular Detection of *K. pneumoniae*: Genomic DNA was extracted from the blood sample of Thalassemia patients isolates by using a DNA Extraction kit (Favorgen \ Austria). All bacterial isolates were subjected to the detection of 16S rRNA (universal gene) by conventional PCR technique using specific primer pairs for every gene
The PCR product of the thermo: The PCR product of nine K. pneumoniae isolated from Thalassemia patients were subjected to partial sequencing of 16S rRNA gene and blasted in NCBI against standard strains of K. pneumoniae. The samples sequences assigned as (OQ928953.1.OQ929666.1.OQ928950.1.OQ929250.1.OQ92 7231.1.OQ928942.1.OQ927249.1.OQ927229.1,and OQ927253.1). A phylogenetic tree for genes sequenced was constructed by using (MEGA10) [11].

<table>
<thead>
<tr>
<th>Primer</th>
<th>5-Sequence -3</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Universal gene F</td>
<td>AGAAGTTGATCCTGGCCTCAG</td>
<td>[12]</td>
</tr>
<tr>
<td>R</td>
<td>GGTATTAGTTACGACTT</td>
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Ethical permission: The study has been approved by Thi-Qar Health Directorate via their agreement code 300/2022.

III. RESULTS & DISCUSSION

From a total of 40 Thalassemia patients' blood samples from both gender, 37(92.5%) samples were given a positive bacterial results, were diagnosed by conventional PCR technique through the amplification of 16S rRNA. As shown in Table (2), the results showed a significant predominance of Gram negative in comparison to Gram positive bacteria with an occurrences of 33(89.2%) and 4(10.8%), respectively (p≤0.001).

As shown in Figure (1), in gram negative bacteria, the most prevalent isolate was K. pneumoniae with a recovery percentage of 9(24.3%), followed by E. coli and p. aeruginosa. with a percentage of 7(18.9%) and (13.5%) respectively. The result of the present study showed that Gram positive bacteria were less frequent with a percentages of 2(5.4%) for Enterococcus sp., where both Staphylococcus aureus and Macroccoccus sp. found in single isolate (2.7%) (p≤0.001).

Infections are major complications in patients with Thalassemia, especially those with thalassemia major. Iron overload, splenectomy, transfusion-related infection [13,14]. One of the main causes of contamination of blood in patients with Thalassemia are Splenectomy process as a result of the spleen amplified. Also, blood transfusions may be lead to contamination and cause septicemia [15]. Thalassemia and sickle cell disease patients to severe infections mostly by Gram-negative bacteria [16]. The major causative organisms in the series from the Far East are Gram-negative bacilli, especially K. pneumoniae (particularly found in liver abscesses). The different spectrum of infections among our patients may be related to better chelation, which makes them less vulnerable to organisms with increased virulence in the presence of excess iron (Y. enterocolitica, K. pneumoniae, E. coli, Str. pneumoniae and L. monocytogenes [17,18]. Similar local and regional studies were in agreement with the present study results that refer to the predominance of Gram negative species in Thalassemia patients, especially K. pneumoniae [19,8].

Detection of 16S rRNA: All isolates (n=37) were diagnosed by conventional PCR technique through the amplification of 16S rRNA genes to confirm that the verified isolates are K. pneumoniae and other bacterial spp. The results showed that all isolates were positive for both the targeted genes. The ladder (3000bp-100bp), the size of products were approximately 1500 bp for 16S rRNA gene. These results agreed completely with previous studies like [12],[20],[21]. The method should allow prompt and accurate identification of bacteria [20]. Gel electrophoresis for 16S rRNA gene after staining with ethidium bromide[21].

Phylogenetic analysis: The nine selected K. pneumoniae strain granted the official Gene bank accession numbers of (OQ928953.1.OQ929666.1.OQ928950.1.OQ929250.1.OQ92 7231.1.OQ928942.1.OQ927249.1.OQ927229.1,and OQ927253.1). The phylogenetic tree showed that there were different molecular relationships among the local K. pneumonia isolates with analogous ones around the world (Fig. 2).

![Figure 1: Percentages of bacterial type isolated from blood directly by PCR.](image-url)
The Sequencing technique is one of the modern advanced development technique in molecular biology. In this way mutation and genetic relationship can be detected between bacterial isolates rapidly [22,23,24,25]. The DNA sequencing analysis results for 16S rRNA K. pneumoniae gene isolates were genetically identical to those found in the gene bank. Nine isolates are shown for the 16S rRNA gene genetically far from the genes taken from the gene bank because they appeared in the out-group. The constructed phylogenetic tree showed that the local K. pneumoniae strains OQ927250, OQ927251, OQ928942, OQ927249 were highly relative to each other in comparison with other related isolates assigned in Iraq and other parts of the world.

IV. CONCLUSIONS

Thalassemia continues to be one of the most socio health problems that need a lot of diagnosis and treatment. Molecular techniques in bacterial identification may contribute as an accurate diagnostic tool for associated infections.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES


