University of Thi-Qar Journal of Science (UTJsci)
E-ISSN:2709-0256, ISSN Print: 1991-8690 Volume (10), No.1, June. 2023

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 ofOQ928953.1,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, phylogenic 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]. 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

https://doi.org/10.32792/utq/utjsci/v10i1.1052

(Table1). The amplification genes were put into the thermo cycler (Hamburg, Germany) and the right PCR cycling program parameters conditions were adjusted according to the primer. Thermal cycling was as follows: denaturation at 94 ºC for 1 min, annealing at 55ºC for 1.5 min, and extension at 72 ºC for 3min for a total of 35 cycle[12, 21].

Sequencing Analysis: 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,OQ927231.1,OQ928942.1,OQ927249.1,OQ927229.1,and OQ927253.1). A phylogenetic tree for genes sequenced was constructed by using (MEGA10) [11].

TABLE **(1):** Sequences of primer.

Primer	5-Sequence -3		Reference
Universal gene	F	AGAGTTTGATCCTGGCTCAG	[12]
	R	GGTTACCTTACGACTT	

Ethical permission: The study has been approved by Thi-Qar Health Directorate via their agreement code 300/2022.

III. RESULTS & DISUSSION

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 ($p \leq 0.001$).

TABLE **(2):** Types of isolated bacteria.

Bacterial type	No.(%)
Gram positive	4(10.8%)
Gram negative	33(89.2%)
Total	37(100%)

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 *Macrococcus* sp. found in single isolate (2.7%) ($p \leq 0.001$).

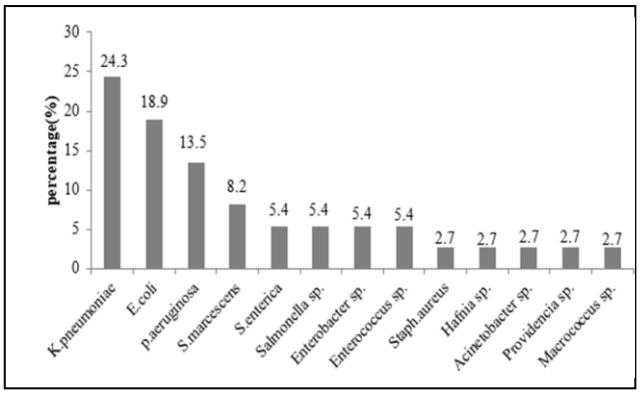

Figure 1: Percentages of bacterial type isolated from blood directly by PCR.

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 predomenance of Gram negative species in Thalssemia 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,OQ927231.1,OQ928942.1,OQ927249.1,OQ927229.1andOQ927253.1).The phylogenetic tree showed that there were different molecular relationships among the local *K. pneumonia* isolates with analogous ones around the world (Fig. 2).

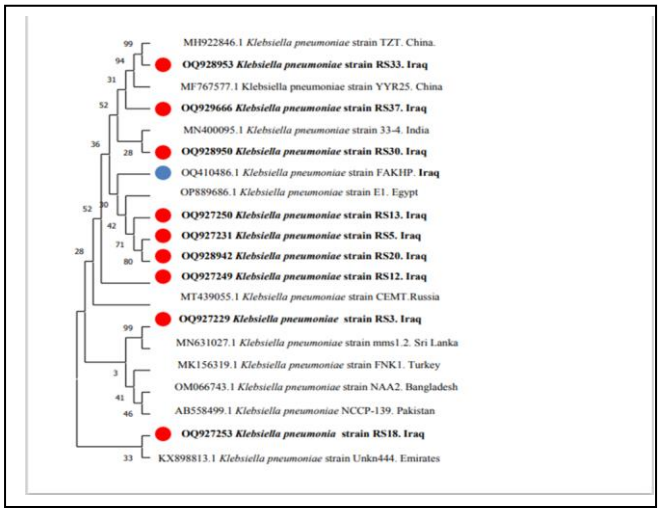

Figure 2: Dendrogram showing the neighbor joining phylogenetic tree analysis based on (16s RNA) gene relationship analysis of local *K. pneumoniae* isolates and related strains from Genebank.

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, OQ927231, 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 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 INTREREST

Authors declare that they have no conflict of interest.

REFERENCES

[1] M.R. El-Shanshory, L. M. Sherief, H. M. Hassab, S. M. Ragab, S. Yahia, A. K. Mansour, & S. M. Saied, "Prevalence of iron deficiency anemia and beta thalassemia carriers among relatives of beta thalassemia patients in Nile Delta region, Egypt: a multicenter study", *Journal of the Egyptian Public Health Association*, *96*, 1-8, 2021.

[2] A. M. Mohammed, "Serum levels of lipid and lipoproteins in patients with beta-thalassemia in Amara S. Iraq", *University of Thi-Qar Journal of Science*, *1*(3), 3-11, 2009.

[3] S. A. Patel, A. M. Siddiqui, & I. Kareem, ''Correlative study of serum bilirubin and liver enzymes with serum ferritin in beta thalassaemia major", *IOSR Journal of Dentaland Medical Sciences*, 17(2), 62-67, 2018.

[4] D. K. Al-Moussawi, "Correlation of HCV Infection and Creatinine Levels in Thalassemia Patients", *University of Thi-Qar Journal of Science*, 9(2), 80-83, 2022.

[5] S. R. Ali, S. S. Sinthee, M. R. Islam, & A. S. Sarwar, "Clinical and molecular studies on thalassemia", *Int J Cur Res Rev*, 10(4), 34-39, 2018.

[6] O. M. Hamed, R. A. Al-Taii, & M. H. Jankeer, "Biochemical and Genetic Study in Blood of β–Thalassemia Children in Mosul City, Iraq," *Iraqi Journal of Science*, 2501-2508, 2021.

[7] A. A. Yasir, "Phylogenetic Groups of *Escherichia coli* Strains from Urinary Tract Infection of Thalassemic Patients in Thi-Qar", M.Sc. Thesis, College of Science / Thi-Qar University, 2020.

[8] F. M. Alzahrani, & S. Sattar Shaikh, "*Acinetobacter baumannii* infection in transfusion dependent thalassemia patients with sepsis", *BioMed Research International*, 2017.

[9] S. Shang, Z. Chen, and X. Yu, ''Detection of bacterial DNA by PCR and reverse hybridization in the 16S rRNA gene with particular reference to neonatal septicemia", Acta Paediatrica, 90(2), 179-183, 2001.

[10] A. Fernández-Olmos, M. I. Morosini, A. Lamas, L. Máiz, and R. Cantón, '' Maldi-Tof MS improves routine identification of non-fermenting Gram negative isolates from cystic fibrosis patients". J. Cystic Fibros., 11(4): 59-62, 2012.

[11] S. Kumar, G. Stecher, M. Li, C. Knyaz, & K. Tamura, ''MEGA X: molecular evolutionary genetics analysis across computing platforms", *Molecular biology and evolution*, *35*(6), 1547, 2018

[12] E. F. DeLong, "Archaea in coastal marine environments", *Proceedings of the National Academy of Sciences*, *89*(12), 5685-5689,1992.

[13] B. J. Al-Badry, "Prevalence of anti-HBV antibodies in multi-transfused patients with thalassemia at Thi-Qar province", *University of Thi-Qar Journal of Science*, *4*(3), 14-17, 2014.

[14] J. M. Sheen, F. J. Lin, Y. H. Yang, & K. C. Kuo, "Increased non-typhoidal *Salmonella* hospitalizations in transfusion-naïve thalassemia children: a nationwide population-based cohort study", *Pediatric research*, *91*(7), 1858-1863, 2022.

[15] B. J. Badry, A. A. Hussin, I.N. Abid, & A. Sh, "Bacterial Infections in Thalassemia Patients at Thi-Qar Province/South Iraq", *American Scientific Research Journal for Engineering, Technology, and Sciences* , *19*(1), 199-205, 2016.

[16] W. Sakran, C. Levin, Y. Kenes, R. Colodner, & A. Koren, "Clinical spectrum of serious bacterial infections among splenectomized patients with hemoglobinopathies in Israel a 37-year follow-up study", *Infection*, 40, 35-39, 2012.

[17] G. Rahav, V. Volach, M. Shapiro, D. Rund, E. A. Rachmilewitz, & A. Goldfarb, ''Severe infections in

thalassaemic patients: prevalence and predisposing factors". *British journal of haematology*, *133*(6), 667-674, 2006.

[18] S. S. Hamim, "Amplification and sequencing of *hla* and *sea* genes in *Staphylococcus aureus* isolated from outpatients in Nassyriah City," *Journal of College of Education for pure sciences*, 7(2), 192-200, 2017.

[19] A. Pishtiwan, & K. Khadija, "Molecular identification of clinical microbes in thalassemia patients using 16S rRNA gene sequencing", *Applied Ecology and Environmental Research*, *17*(6), 13135-13146, 2019.

[20] T. Miyoshi, T. Iwatsuki, & T. Naganuma, ''Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer-pore-sizefilters", *Applied and environmental microbiology*, *71*(2), 1084-1088, 2005

[21] S. A. Barghouthi, "A universal method for the identification of bacteria based on general PCR primers", *Indian journal of microbiology*, *51*, 430-444, 2011.

[22] K. Tamura, G. Stecher, D. Peterson, A.Filipski, & S. Kumar, ''MEGA6: molecular evolutionary genetics analysis version 6.0", *Molecular biology and evolution*, *30*(12), 2725-2729, 2013.

[23] A. R. Ahmed & S. S. Hamim, "Phylogenetic Profile of *Staphylococcus aureus* mec A and ICA a Genes Associated with UTI Patients", *Indian Journal of Public Health Research & Development*, 11(2), 1169-1174, 2020.

[24] M. G. Hassan, Z. R. Hassan, S. S. Hamim, M. O. Abdel-Monem, S. A. Abo-Elmaaty, "Antimicrobial and antibiofilm activity of *Streptomyces* sp.AS5 against *Klebsiella pneumonia* and *Escherichia coli*", Chin J Ind Hyg Occup Dis, 13(40), 33-41, 2022.

[25] S. S. Hamim, "Molecular characterization of *mec*A gene in Methicillin-Resistant *Staphylococcus aureus*", *University of Thi-Qar Journal of Science*, *6*(1), 25-29, 2016.