Molecular and Phylogenetic study of *Klebsiella aerogenes* isolated from infected burned skin

Alaa Abdelkadhim Jawad[(1)] , Hayder Naji Ayyez[(1)] and Hayder Ali Muhammid[(2)]

[1] Unit of Zoonosis , College of Veterinary Medicine, University of Al-Qadisiyah,

[2] Department of Microbiology, College of Vet. Medicine, Kerbala University,

Email: alaa.jawad@qu.edu.iq

Abstract

This study was intended to explore if infected burns of patients of Al-Diwaniyah City, Iraq, have *Klebsiella aerogenes* as one of the pathogenic bacteria that infect burns or wounds. For such case, traditional, Polymerase chain reaction (PCR), and DNA gyrase subunit B gene (*gyrB*)-partial sequencing tooling to confirm diagnosis. Primary testing of the sample using blood and MacConkey agars and certain biochemical tests alarmed that the bacterium was present in this specimen. The PCR results confirmed the occurrence of the infection by this bacterial agent. The *gyrB*gene-partial sequencing followed by drawing a phylogenic tree placed the bacterium in a separate cluster from other Enterobacteriaceae family members. The current study indicates that this bacterium is present in the infected burns or injuries in Al-Diwaniyah city. Our results have been reviewed the current trends in molecular phylogenetic analysis, A computational phylogenetic study and data was approach and registered *Klebsiella aerogenes* with accession number (MG560868.1).

Keywords: *Klebsiella aerogenes*, PCR, Sequencing, Phylogeny, Burn infections.

Introduction

The infected burns are considered as a major problem that could be acquired most of the times in hospitals which leads in some cases to severe infection that could be ended with death of patients **[1]**. In hospitals, using not clean or non-sterile tools could leave burned skin of patients entering hospitals seeking for medical assistance prone to ugly bacteria**[2]**. In some cases, even the use of highly hygienic methods in cleaning and disinfecting of hospital facilities may still have dangerous bacteria or their spores that could affect patients in these places, so the use of different sterilizing liquids could overcome this resistance problems**[3]**. Wide range of bacteria isolated from infected burnt skin of people entered healthcare centers, and most of these bacteria could be thrived in these centers after dealing with different infectious cases. These bacteria could range from gram negative and positive, fecal, and various infectious bacteria **[4]**. One of the annoying infectious bacteria that affect burn wounds is *Klebsiella* spp. that is one of the most bacteria isolated from infected burns in the healthcare facilities **[5]**. In Iraq, This the first study, according to our information, that identifies *Klebsiella aerogenes*, which is also known as *Enterobacter aerogenes* **[6]** in infected burns using various tools including molecular and phylogenetic assays.

Materials and methods

Sampling and cultivation

Using sterile cotton swabs, 150 samples were directly cultivated on blood and MacConkey agars for 24 hrs **[7]**. The growing colonies was subjected to staining and preliminary identified Gram positive and Gram negative bacteria were subjected for colorimetric identification by Vitek^{TM}2 compact system according to the instruction provided by the company. The results of this test were automatically interpreted via the ID-GPC library.

Five colonies of overnight cultures were mixed in normal saline to give turbidity equal to 0.5 Macfarland standard in special tubes provided with the vitek system. The bacterial suspension in these tubes will bushed to special kit in Vitek ^{TM} 2 compact system to colorimetric identification of bacterial isolates.

DNA extraction and polymerase chain reaction (PCR)

The bacterial DNA was extracted using GB100 kit that had been purchased from Geneaid Company, Taiwan. The manufacturer protocol was followed to extract the DNA. The primers used to amplify a 1256-sized piece of *gyrB*gene are 5'GAA GTC ATC ATG ACC GTT CTG CA3' and 5'AGC AGG GTA CGG ATG TGC GAG CC 3' **[8]**. The PCR mastermix was prepared using GoTaq Green mastermix kit which had been purchased from Promega Company, USA. PCR reaction mixture were 12.5 for Green mastermix **,** 1.25 µ for each forward and reverse primers , 5 µ for DNA

template and complete the volume by adding 5 µ of nuclease free water. The thermocycler conditions used for the amplification of this piece were Initial Denaturation 95C for240 sec then 30 cycles from 94C for60 sec , 64C for 40sec and 72C for 60 then72C, 600sec for Final extension. The products were tested using agarose 1% gel electrophoresis and explored using UV imager.

GyrB partial sequencing

The piece of the gene was sequenced at Macrogen Company, South Korea. The sequence processing was done using NCBI- Blast and aligned with some known species of other Enterobacteriaceae members. The phylogenic tree was generated using Mega v6 software depending on the Neighbor-Joining method. The distances were calculated by the Maximum Composite Likelihood method**[9 ; 10]**

Results:

Cultivation and the biochemical tests:

According to cultural characteristic and Vitec ^{TM}2 compact system the results indicated the presence of the *Klebsiella aerogenes* in the tested samples which later confirmed by the PCR.

Polymerase chain reaction (PCR): The results showed amplification of the 1256-sized piece of the *gyrB* gene. The amplification image is shown in figure 1.

***GyrB* partial sequencing and the phylogenic tree:** the results revealed that the bacterium was an isolate of *Klebsiella aerogenes* bacterium. When it is compared with other known isolates of other enterobacterecae family members, it showed distinct clustering on the drawn tree, figure 2.

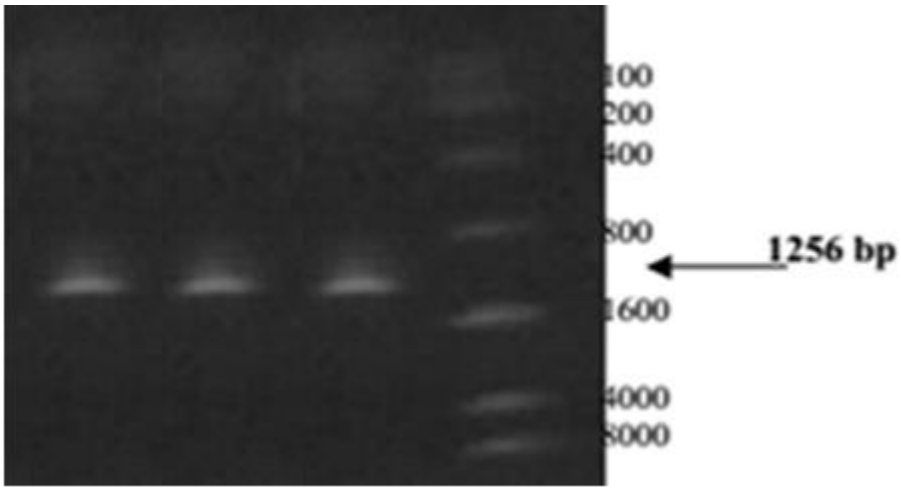

Figure 1: PCR amplification of the *GyrB* at 1256Bp. Ladder is 8000-100bp.

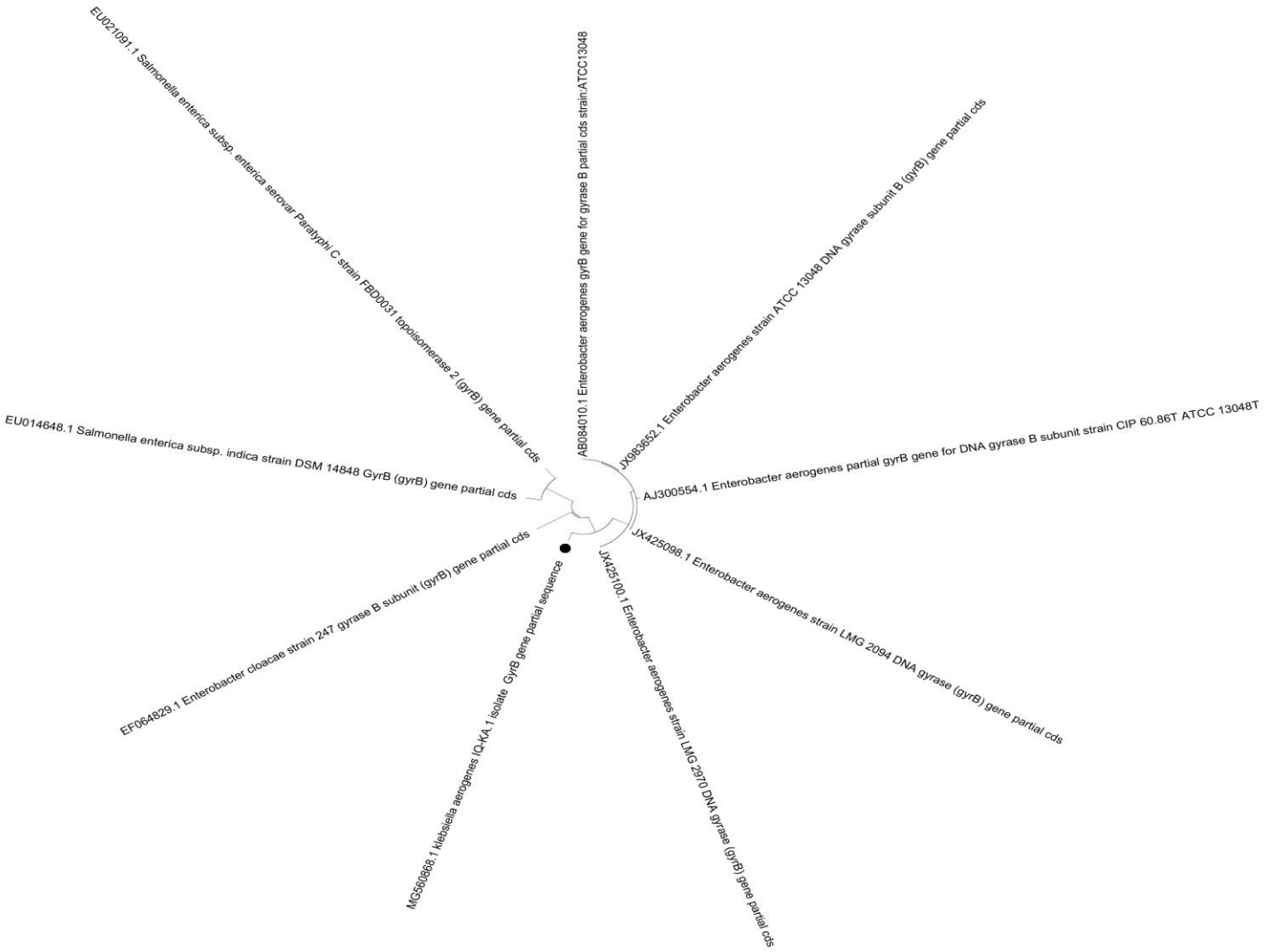

Figure 2: shows the distinct clustering of the current study isolate that is represented, here, by the black dot.

Discussion

The collected results referred to the actual presence of *Klebsiella aerogenes* in the burns, and this indicated that this bacterium might have come to the affected wounds from the healthcare center facilities. The primary test showed that the burns were infected with this bacterium, and this agrees with **[11]** who mentioned the isolation of this bacterium from aspirate of trachea of a senior gentleman. The normal place or habitat for *Klebsiella aerogenes* is the wards of hospitals **[12]**, and this allows the bacterium to infect wounds or burns. This also confirms the current study results that this bacterium was acquired in the hospital, and the infections might have been only occurred in the hospital where the samples were collected from. From the above mentioned literatures, the bacterium had been isolated from the tracheal component, and this gives the idea that contamination of the hospital was from either respiratory infections of worker people, patients, or both. The PCR results assured the primary test results and gave better understanding of the risky presence of this infectious bacterium in the burns. This agrees with **[13]** who isolated the bacterium from fecal and blood samples of infants in a healthcare unit. This initiates the opinion that this bacterium is also could thrive in hospitals and receive this contamination from feces and blood. This bacterium clustered in a distinct branch in the phylogenic tree that was drawn for this study, and this indicates that the bacterium detected is *Klebsiella aerogenes* but not any other member of the Enterobacteriaceae family

member **[14]**. This provides evidence that this bacterium might have acquired the resistibility status which allows it to thrive in hospitals, and this risk needs further future studies to increase knowledge of fighting this bacterium.

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