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Original Article

Molecular Detection of *bla*$_{SHV-la}$ Gene in *Klebsiella pneumonia* Isolated from Urinary Tract Infections, Najaf, Iraq

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Abstract

Klebsiella pneumoniae is a gram-negative bacterium that causes serious illnesses, including pneumonia, liver abscess, meningitis, bloodstream infections, and urinary tract infections (UTIs). This study aimed to isolate and diagnose *K. pneumoniae* from clinical specimens of urine from patients with UTIs and perform molecular detection of the *bla*$_{SHV-la}$ gene in *K. pneumonia* in the Najaf Province, Iraq. The study included 100 clinical specimens from October 2021 to March 2022. As an initial diagnosis, *K. pneumoniae* isolates were diagnosed based on culture and biochemical features. Apart from the usage of polymerase chain reaction (PCR) technology to identify the *bla*$_{SHV-la}$ gene, the final diagnostic was achieved by the automated Vitek-2 compact system. The biochemical findings revealed that 40 out of every 100 isolates tested positive for *K. pneumoniae*. These results were validated by Vitek, which revealed that 40/100 of the samples tested positive for *K. pneumoniae*, and by PCR utilizing the *bla*$_{SHV-la}$ gene, which showed that 13/40 of the samples tested positive for *K. pneumoniae* isolated from the urine of patients with UTIs. In conclusion, the results indicated that the use of the Vitek-2 technique was required to confirm the accurate identification of the pathogen. *Klebsiella pneumoniae* clinical isolates showed multidrug resistance to antibiotics commonly used to treat UTIs. The *bla*$_{SHV}$ gene encoded for Extended-spectrum beta-lactam antibiotic was found almost in *K. pneumoniae* isolates.

Keywords: *Klebsiella pneumoniae*, *bla*$_{SHV-la}$ gene, Urinary tract

1. Introduction

Klebsiella pneumoniae is a gram-negative bacterium that causes serious illnesses, including pneumonia, liver abscess, meningitis, bloodstream infections (BSIs), and urinary tract infections (UTIs). Because of the high likelihood of acquiring multidrug resistance (MDR), treatment options for these diseases are severely restricted (1). *Klebsiella* bacteremia and pneumonia are caused by both community-acquired and surgical site infections, in accordance with the American Society of Microbiology. *Klebsiella pneumoniae* has been associated with a 20-50% mortality rate and a 50% chance of developing pneumonia. *Klebsiella* infection is a key contributor to the development of septicemia and bacteremia in children and adults hospitalized in intensive care units. It is believed that the pathogenesis of this bacteria is aided by a variety of virulence factors, such as adhesion, siderophore, O antigen, and the capsule (2). It is commonly assumed that *K. pneumoniae* is an opportunistic pathogen, meaning that it can cause infections in immunocompromised individuals and lead to UTIs, pneumonia, and BSIs (3). *Klebsiella pneumoniae* is a gram-negative bacterium that is the second most cause of BSIs. Antibiotic

resistance genes have made it difficult to treat *K. pneumoniae* infections, and multidrug-resistant *Klebsiella* spp. have now been identified as a public health issue, particularly for vulnerable individuals (4). Extended-spectrum beta-lactamases (ESBLs) refer to bacterial enzymes that provide resistance to a wide range of β-lactam antibiotic classes in bacteria (5). SHV-lactamases are found in abundance in gram-negative bacteria. SHV-1 is capable of hydrolyzing penicillin and cephalosporin antibiotics. The invention and popular usage of broad-spectrum antibiotics have led to the emergence of ESBLs, which can hydrolyze these drugs (6).

This study aimed to isolate and diagnose *K. pneumoniae* from the clinical specimens of urine from patients with UTIs and perform molecular detection of the *bla*$_{\text{SHV-la}}$ gene in *K. pneumonia* in the Najaf Province, Iraq.

2. Materials and Methods

2.1. Collection of Specimens and Identification of Bacteria

Urine samples (n=100) were collected from patients with urinary tract disorders at various hospitals in Najaf from October 2021 to March 2022. Urine samples were cultured at 37°C for 18-24 h after being cultivated on MacConkey agar and Blood agar. After staining with gram-stain and performing a biochemical test for *K. pneumoniae* identification by examining colony attributes, such as size, color, boundaries, and texture, it was determined that the bacterium was *K. pneumoniae*. Finally, the identification was accomplished with the help of the automated Vitek-2 compact system and G-ve ID cards.

2.2. Molecular Investigation

2.2.1. Genomic DNA Extraction

The extraction of genomic DNA was performed by boiling method. In order to lyse the bacteria cultured on MacConkey agar plates and release their DNA, the isolated colony were vortexed and warmed up to 100°C for 15 min in an Eppendorf tube, and then frozen at -20°C for 20 min to lyse the bacteria and release their DNA from the plate. Afterward, the DNA was centrifuged at 8000×g for 5 min before transfer to Eppendorf tubes and kept at -20°C for next usage.

2.2.2. Identification at the Molecular Level

An ultraviolet transilluminator (Cleaver, UK) was employed to identify DNA using gel electrophoresis. The *bla*$_{\text{SHV-la}}$ genes for *K. pneumoniae* were explored utilizing a polymerase chain reaction (PCR) test, as indicated in table 1. According to the information in table 2, this primer was progressed by the Alpha DNA Company in Canada. To validate the amplified products, the size of the PCR products was determined using 0.8% agarose gel electrophoresis. An ethidium bromide solution (4 µL of 10 mg/mL) was used to stain the gel, which was operated at 70 v for 1.5 h (Sigma, USA). On the ultraviolet transilluminator (Cleaver, UK), a single band was observed at the appropriate spot; the bands were photographed using a gel documentation system (Cleaver, UK). Measurement of the molecular weights of amplified products was carried out using a 100bp ladder (Bioneer, Korea).

Table 1. Thermocycler-based PCR protocol for the *bla*$_{\text{SHV-la}}$ primer

Gene	First denaturation	No of cycles	Denaturation	Annealing	Extension	Last extension
bla$_{\text{SHV-la}}$	95°C for 5 min	40	95°C for 1 min	50°C for 1 min	72°C for 2 min	72°C for 7 min

Table 2. Primers utilized in the current investigation

Kind of primer	Sequence of primer (5'-3')	Amplicon size (bp)
bla$_{\text{SHV-la}}$	Forward, 5'- ATCTGGTGGACTACTCGC- 3' Reverse, 5'- GCCTCATTCAGTTCCGTT- 3'	213

3. Results and Discussion

3.1. Prevalence of *K. pneumoniae* Specimens and Morphological Characterization

In this investigation, 40 urine specimens from patients with UTIs were used from October 2020 to March 2021. On MacConkey agar and Blood agar, 100 specimens were cultured and incubated at 37°C for 18-24 h. The bacterial isolates from clinical samples were initially identified by their culture morphology, microscopic features, and biochemical properties. The colonial morphology of *K. pneumoniae* isolates was used to determine the species of bacteria from which they were isolated. The fact that *K. pneumoniae* produces non-hemolytic smooth white colonies on blood agar and red color and smooth colonies on MacConkey agar indicated that *K. pneumoniae* might ferment lactose sugar (Figure 1). As a supplement to the initial identification of *K. pneumoniae* isolates, the findings of biochemical tests were judged to be useful. The isolates were found to be negative for the oxidase test, which confirmed the overall features. The synthesis of urease, the usage of Simmon citrate, and the catalase test all yielded positive results. All results (morphology and culture) were the same as the original. There were 40 isolates that were provisionally recognized as *K. pneumoniae* after a thorough bacteriological examination based on the morphological, cultural, and biochemical tests. The automated Vitek-2 compact system completed the process using GN-ID cards with 47 biochemical tests and 1 negative control well. The findings showed that 40 isolates from patients with UTIs were verified as *K. pneumoniae*, with an ID message confidence level ranging from very high to outstanding (99% probability). This result was in agreement with that reported in a study by Al-Kraety, Alquraishi (7), who conducted research on bacterial diagnostics and determined that the compact Vitek-2 automated approach was effective.

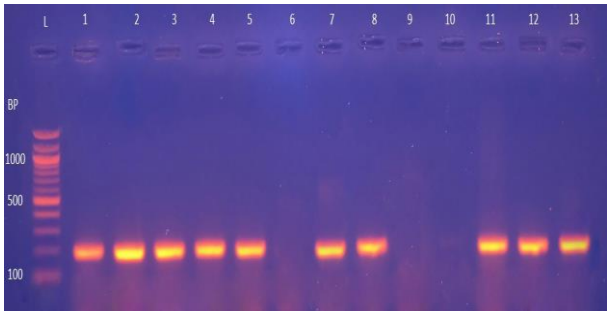

Figure 1. PCR amplicon of *K. pneumoniae* bla_{SHV} gene
L: Lane; Product size: 213bp. DNA marker: 100-bp ladder; Lanes 1-5, 7, 8, 11-14: positive results; Lanes: 6, 9, 10: negative results

3.2. Molecular Detection of $bla_{SHV\text{-}la}$ Gene of *Klebsiella pneumoniae*

The amplification findings of the PCR research for bla_{SHV} (213bp) indicated that 13 (35%) of *K. pneumoniae* isolates tested positive for the bla_{SHV} gene, as depicted in figure 1. Genetically modified bacteria carry self-transmissible conjugative plasmids with ESBL enzymes ($bla_{CTX\text{-}M}$, bla_{TEM}, and $bla_{SHV\text{-}la}$) (8). These inhibitors prevent the synthesis of ESBLs, such as clavulanic acid and tazobactam, which may be used to identify ESBLs in the Enterobacteriaceae family, such as *Escherichia coli*, *K. pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis*. Molecular-based techniques, including multiplex PCR assays, are more suitable for ESBL testing for epidemiological comparison or infection control (9). The bacterium *K. pneumoniae* causes several ailments, including nosocomial and community-acquired ones. Beta-lactam antibiotics are extensively used to treat *K. pneumoniae* infections, which has led to severe problems in medical clinics during the last two decades (10).

The results of a study by Kumar, Sun (11) reported *K. pneumoniae* to be MDR to fluoroquinolones, aminoglycosides, trimethoprim, and sulfamethoxazoles, which was in line with our findings. The genetic and phenotypic diversity of clinical isolates, additional efflux pumps, and multiple fluoroquinolone resistance pathways all contribute to antibiotic resistance in bacteria (10).

The Vitek-2 technique was required to confirm the pathogen's accurate identification. *Klebsiella pneumoniae* clinical isolates showed multidrug resistance to antibiotics commonly used to treat UTIs. The bla_{SHV} gene encoded for ESBL antibiotic was found almost in *K. pneumoniae* isolates.

Authors' Contribution

Study concept and design: M. R. M.
Acquisition of data: D. A. H. K.
Analysis and interpretation of data: S. S. A. A.
Drafting of the manuscript: B. A. A.
Critical revision of the manuscript for important intellectual content: A. F. A. and M. R. M.
Statistical analysis: M. R. M. and B. A. A.
Administrative, technical, and material support: M. R. M. and D. A. H. K.

Ethics

The study design was approved by the ethics committee of the Islamic University, Najaf, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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