Molecular Study of *Fimh* Gene in *Klebisella Pneumonia*e Isolated From Urinary Catheter Patients

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Abstract

This study aimed to isolate and diagnose *K. pneumoniae* from clinical specimens of urine from urinary catheterized patients and molecular Detection of *FimH* fimbrial adhesin in *Klebisella pneumonia* in Najaf governorate from October 2018 to March 2019, which includes 40 clinical specimens (urine). The diagnosis of *K. pneumoniae* isolates was based on culture and biochemical characteristics as an initial diagnosis. The final diagnosis by the Vitek-2 compact system is automated besides the use of PCR technique to detect on fimH fimbrial adhesion gene.

The biochemical results showed that 40/40 isolates gave positive result of *K. pneumoniae*.These results were confirmed by Vitek showed that 40/40 were positive for *K. pneumoniae* isolated and PCR technique by using *fimH* gene where 14/40 were positive for *K. pneumoniae* isolated from urine urinary catheterized patients.

The study, which was conducted in the diagnosis of bacteria, concluded that the technique of compact Vitek-2 automated. The ability of bacteria to stick to the formation of biofilm was investigated by phenotypic method.

***Keywords**: K. pneumonia, fimH gene, urinary catheterized.*

Introduction

Catheter-associated urinary tract infections (CAUTIs) are most frequent as a nosocomial infection with increased patient morbidity and health care costs. *Klebsiella pneumoniae* is a prominent opportunistic pathogen causing infection in 10% of the patients with urinary catheters. The catheter insertion provides site for bacteria attachment that is typical in Gram-negative enterobacteria [(1, 2)]. Furthermore, about 30% of *K. pneumoniae* isolates are resistant to broad-spectrum antibiotics with many virulence factors that have been identified . Fimbrial adhesins play an important role in the bacteria pathogenicity that facilitate adherence to specific tissue surfaces. Type 1 fimbriae, especially FimH subunit, found in many members of Enterobacteriaceae and play an important role in UTI [(3-5)]. Fimbriae are encoded by Fim gene cluster containing all fimbrial structure genes coding for repeating FimA subunits with an adhesin molecule (FimH) at the tip [(6)].

Material and Method

Patients and clinical specimens

A total of 40 urine samples were collected from urinary catheterized patients from different hospitals in Al- Najaf provenance from Oct. 2018 to March 2019.

Urine specimens were cultured on MacConkey and Blood agar figure (1), then inoculated at 37°C for 18-24 hours [(7)].

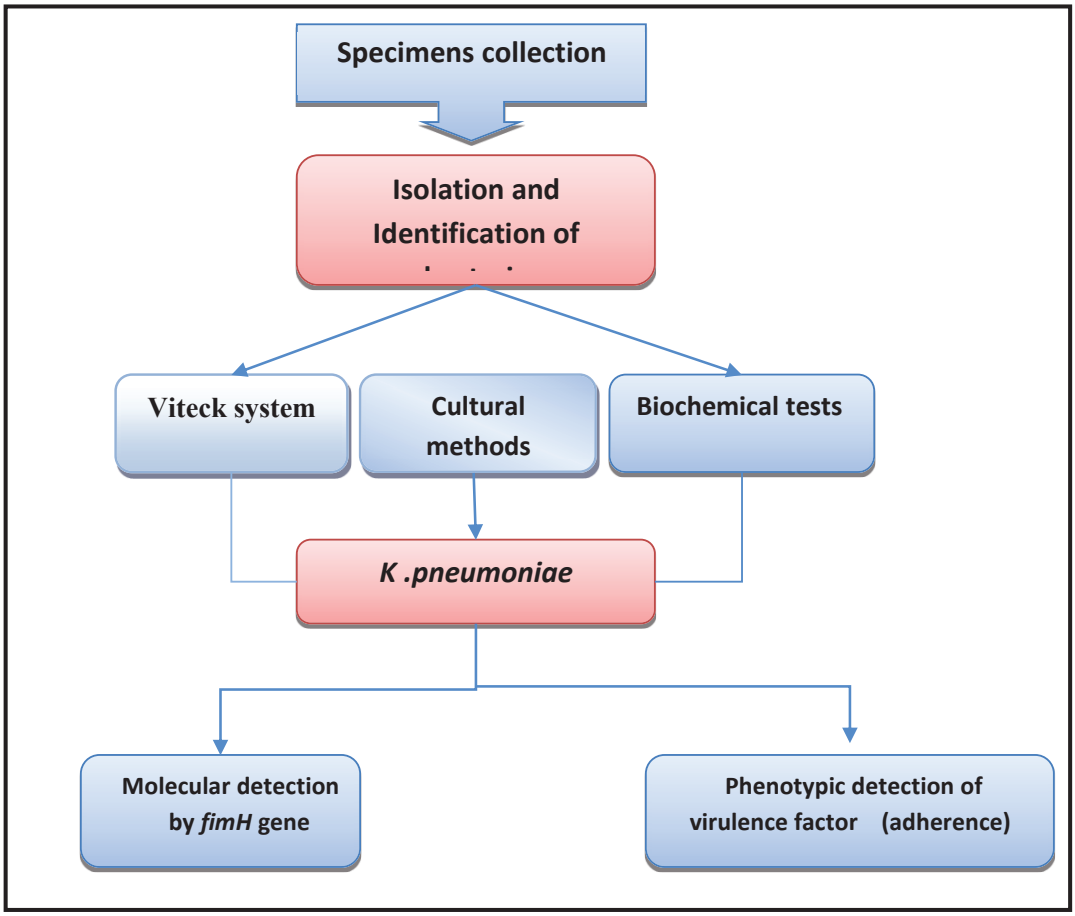

Figure 1: Scheme of the current study

Identification of bacteria

It studied the colonial characteristics such as (shape, volume, color, borders and texture) and examined microscopically after staining with gram-stain [8] and biochemical test for diagnosis of *K. pneumoniae*. Finally, identification was performed with automated VITEK-2 compact system using G-ve ID cards.

Molecular study

Extraction of Genomic DNA

Genomic DNA was extracted by using boiling method DNA was extracted from colonies grown on agar plates by boiling method according to , taking colonies of bacteria grown on MacConkey agar plates were suspended in 300µl of Tris-EDTA Buffer buffer in Eppendorf tube, then vortex and boiling at 100°C for 15 min and immediately freeze at -20°C for 20 min to lyses the organisms and release the DNA, then centrifuged at 8000 xg for 5 min ,supernatant transferred to new Eppendorf tube and stored at -20°C until used.

Polymerase Chain Reaction Protocol

The DNA extract of *K. pneumoniae*. Isolates were subjected to flageller gene genes listed in (Table 1) by using PCR. The protocol was used depending on Promega Biosystem manufacturer's instruction. Single reaction (final reaction volume 20µl as in table (1). All PCR components were assembled in PCR tube and mixed by refrigerated microcentrifuge at 50 rcf for 10 second.

Table 1: Protocol of monplex PCR reaction mixture volumes

Master mix	8µL
DNA template	5µL
Forward primers	1.5µL
Reverse primers	1.5µL
Deionidied water (d d water)	4µ L
Final volume 20 µL	

PCR Thermocycling Conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in table (2).

Agarose Gel Electrophoresis: According to [9].

Table 2: Amplification Conditions of genes were used by PCR reactions.

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
fimH	94ºC for 4min.	35	94ºC for 30sec.	52.9ºC for 30sec.	72ºC for 50sec.	72ºC for 7min.

Results and Discussion

Prevalence of *K. pneumoniae* specimens

This study was conducted on 40 specimens from urine urinary catheterized patients during the period from October 2018 to March 2019, all these specimens 40 were inoculated on MacConkey and Blood agar medium as at 37°C for 18-24 hours [7].

***K. pneumoniae* identification**

Morphologically characterization

The bacterial isolates obtained from clinical samples were identified initially according to cultural morphology, microscopic characteristics and biochemical tests. From those isolates, the cultural identification of *K. pneumoniae* was depended on the colonial morphology. Since the colonies of *K. pneumoniae* were grown on blood agar appears non-haemolytic smooth white colonies and red colour like shaped and smooth colonies when grown on the MacConkey agar, indicated that *K. pneumoniae* is able to ferment lactose sugar (Figure 4-1).

The results of biochemical tests that recorded in table (3) were considered as a complementary of the initial identification of *K. pneumoniae* isolates. The isolates confirm to general characteristics, isolates were negative for oxidase test . Urease production and Simmon citrate utilization and catalase test positive result. All the result (morphology and cultural) were identical with [10].

Table 3: The Biochemical features of *K. pneumoniae*

Test	Result
Oxidase test	-
Simmons Citrate	+
Urea hydrolysis	+
Catalase test	+

Upon detailed bacteriological investigation based on the morphological, cultural and biochemical tests were 40 isolates as tentatively identified as *K. pneumoniae*.

The final identification was performed with the automated VITEK-2 compact system using GN-ID cards

which contained 47 biochemical tests and one negative control well (Appendix1). The results demonstrate that 40 isolates from urine urinary catheterized patients were confirmed as *K. pneumoniae* with ID message confidence level ranging between very good to excellent (Probability percentage 99).

Virulence factors of *K. pneumoniae*

Adherence Variation

Biofilm forming ability is highly linked to bacteria swarming in, which is represented as an important virulent factor [11, 12]. All of the tested isolates showed (2cm-8cm) swarming (increase in colony diameter) after 24 hrs of incubation. The rates of the migration were measured at 0, 6, 12, 18 and 24 hr. At 0 hr, *K. pneumoniae* isolates appeared with no migration. At 6 hr and 12 hr, *K. pneumoniae* isolates appeared with same rate of migration. After 18-24 hrs, most of the *K. pneumoniae* isolates covered the entire media surface. These results agree with the findings of [12] who reported more than 1.5Cm – 7.7Cm after 24 hrs of incubation.

Molecular Study

Genomic DNA Extraction

DNA Genomic was successfully extracted from *K.pneumoniae* isolates by using boiling method. The concentration and purity of extracted DNA were directly determined by spectrophotometry, extracted DNA purity ranged between (1.8 - 2). Extracted DNA were confirmed and analyzed by gel electrophoresis.

Molecular Detection of *fimH* gene of *K. pneumoniae*

The results showed that *fimH* gene was detected in 14 / 40 of *K. pneumoniae* isolates as in figure (2).

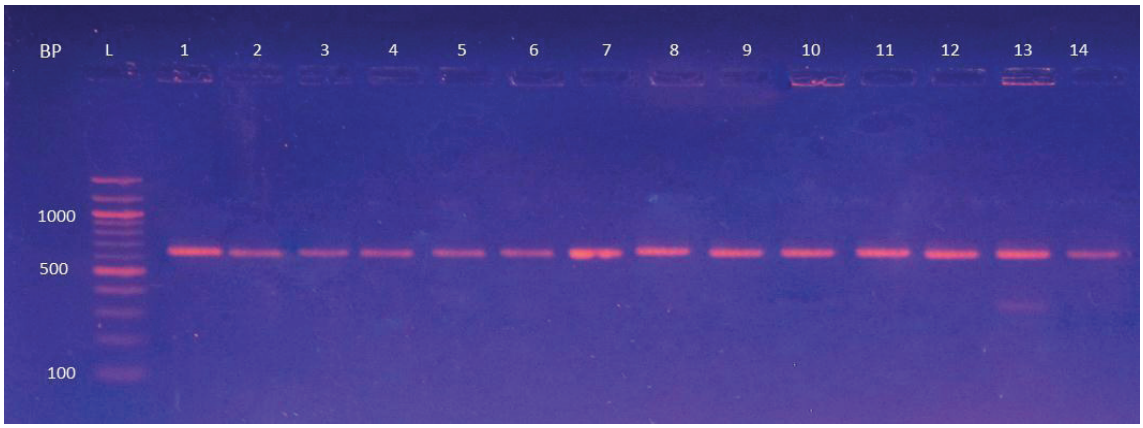

Figure 2: PCR amplicon of *K. pneumoniae fimH* gene. Product size 688bp. Lane (L), DNA marker (100-bp ladder), Lanes (1 to 14) positive results.

Fimbriae are assumed to play critical roles in attachment to epithelial cell surfaces. Binding to specific host receptors, fimbriae mediates the bacterial colonization, host cell signaling. Fimbrial adhesins determine the fate of the bacterial pathogen in the host as well as the progress of the corresponding disease process .Type-1 fimbriae also play an important role in deciding the virulence of the organism. Experiments conducted by Jaroni indicated that a mannose-resistant haemagglutinin was required for the attachment of Klebisella to target cells [13]. The present result assumed important role of fimbriae 1 in attachment to epithelial cell surface (mediates the bacterial colonization) and deciding the virulence of the *K. pneumoniae*. The relationship between mannose – sensitive hemagglutinin (MSHA) or type 1 fimbriae and pathogenisity of bacteria was established from adherence of bacteria in mucous surfaces or epithelial cells of gastric tract [14].

Conclusions

The use of Vitek-2 system, is necessary to confirm precise identification of this pathogen. Clinical isolates of *K. pneumoniae* possess number of virulence factor that associated with Urinary Catheter such as adhesion factors (biofilm). The *fimH* gene that encoded for adhesion factors (biofilm) was found almost in *K. pneumoniae* isolates

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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