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Molecular Detection Of Klebsiella Pneumoniae Isolated From Different Clinical Source

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ABSTRACT

The World Health Organization has listed Klebsiella pneumoniae as one of the global priority pathogens in critical need of next-generation antibiotics. Compared to other Gram-negative pathogens, K. pneumoniae accumulates a greater diversity of antimicrobial-resistant genes at a higher frequency. This study included collecting 60 samples from different sources (wounds, Burns, sputum, urine), and the samples were diagnosed by traditional methods, culturing samples on different media, then conducting biochemical tests, and initial tests showed that 50% of the samples were Klebsiella pneumoniae bacteria, and the rest For species (Escherichia coli 30%, Streptococcus pneumoniae 10%, Pseudomonas aeruginosa 5%, Staphylococcu aureus 5%), then Klebsiella pneumoniae was diagnosed by polymerase chain reaction (PCR) using the diagnostic gene (infB). The results showed that only 15 samples out of 30 had this gene. It was diagnosed as Klebsiella pneumoniae, after that CTX resistance gene was examined, and the results showed the presence of this gene in 67% isolates and its absence in 33%. The results showed the presence of this gene in bacteria isolated from urinary tract infection, and with a small percentage of samples isolated from

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INTRODUCTION

Klebsiella spp. are the causative agent of numerous kinds of infections in humans including: urinary tract infections, respiratory tract infections, and blood stream infections , It is involved in extra-intestinal infections including urinary tract infections, cystitis, pneumoniae,surgical wound infections and life-threatening infections, such as endocarditis and septicemia. It is also an important cause of serious community onset infections such as necrotizing pneumonia, pyogenic liver sores and endogenous endophthalmitis .*Klebsiella* species (spp.) are Gram-negative, non-motile, rod shape, soil and readily isolated from mammalian mucosal surfaces belong to the *Enterobacteriaceae* family. *Klebsiella spp.* are generally found in animal and human gut microbiota , They colonize a wide range of hosts including plants and mammals and can grow ubiquitously in water and soil and *Klebsiella spp.* are generally opportunistic pathogens and do not usually affect healthy individuals (Wyres and Holt, 2018).

Klebsiella spp. utilize the following virulence traits to protect themselves from the host immune response capsular polysaccharides (CPS), lipopolysaccharides (LPS), siderophores, fimbriae (alternatively, pili), a type VI secretion system, outermembrane proteins, porins, efflux pumps, an iron transport system, biofilms, and allantoin metabolism. Among these, CPS, LPS, siderophores, and fimbriae are well characterized virulence factors of *Klebsiella* spp. These virulence factors assist *Klebsiella spp.* in evading the innate immune response of the host and to survive in different sites within the host, rather than actively suppressing host immune system components (Domenico *et al.*, 1994; Hsieh *et al.*, 2019). This report aims to study the characters and virulence factors of *Klebsiella pneumoniae* and Screening of the *CTX* resistance gene from different clinical sources.

Sample Collection:

During the period extended From March 2020 to July 2020 .60 samples were collected from Baghdad city, these samples included: wound swab (11), urine (25), sputum (16) and burns (8)

Bacterial Isolation and Identification:

Bacterial isolates are diagnosed by conventional microbiological methods (colonial morphology, Gram staining, and biochemical tests) according to (Cheesebrough, 1998), and molecular method (PCR).

PCR amplification:

DNA template of all isolates was prepared by boiling method (30 min in 100°C). The DNA of isolates was targeted for the *infB* 1 and gene using the primers listed in Table 1 and for the *CTX* using the primers listed in Table 1.

A reaction mixture (25 µl) contained 2 µl of DNA, 1 µl of each primer, 12.5 µl of Master Mix 2Xand 8.5 µl of Nuclease Free Water. The experiment was continued according to the following program: initial denaturation at 95°C for 5 minutes, followed by 30 cycles at 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec and a final extension at 72°C for 7 minutes. The PCR products were analyzed using gel electrophoresis (1% agarose) and stained with safe dye and visualized by Gel Doc apparatus (BioRad, USA) (Table 2).

Table 2 : PCR program

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	

Annealing	50**or** 55	00:30	30
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Table 1 : Primers used in this study

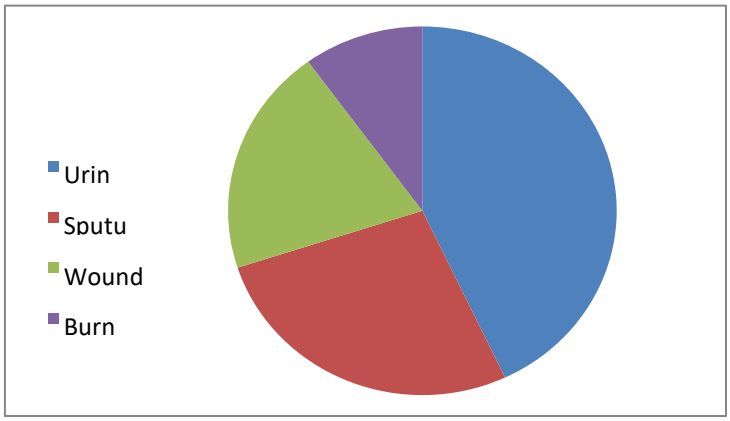
Primer Name	Seq.	Annealing Temp. (˚C)	Product size (bp)
infB1-F	5`-CTCGCTGCTGGACTATAT TCG-`3	55	462
infB1-R	5`-CGCTTTCAGCTCAAGAACTTC-`3	55	462
CTX-F	5`-GACGATGTCACTGGCTGAGC-`3	60	499
CTX-R	5`-AGCCGCCGACGCTAATACA-`3	62	

RESULTS AND DISCUSSION

Characteristic	*Klebsiella pneumoniae*
Gram Staining	-
Shape	Rod
Catalase test	+
Oxidase test	_
Indol Test	_
Methyl Red Test	_
MR-VP Test	+
Citrate Utilization Test	+
Fermentation of Glucose	+
Fermentation of Lactose	+
H2S	_
Gas	+
Urease	+

60 samples were collected and diagnosed in different methods, the results showed that 50% of the samples were for *Klebsiella pneumoniae* distributed, the largest percentage was isolated from urine as the following (43% of urine, 27% of sputum, 20 of wounds, 10% of burns) and the remaining 50% The different bacterial species were (*Escherichia coli* 30%, *Streptococcus pneumoniae* 10%, *Pseudomonas aeruginosa* 5%, *Staphylococcu aureus* 5%).The samples were then subjected to different types of media and biochemical tests to isolate the bacteria (fig. 1).

Figure (1) The percentage of presence of K.pneumonia in different sources



Biochemical test Results in table (3) showed that the isolates gave positive results for catalase due to the ability to produce catalase enzyme (that reduce hydrogen peroxide to water and oxygen gas bubbles), negative results for oxidase due to the inability to produce cytochrome C oxidase (that oxidase tetramethyl-p phenylenediamine), positive results for Citrate utilization due to the citrate considered a sole carbon source. All isolates were negative for indole testing and here is an important point to distinguish between *K.pneumonia* and *klebsiella oxytoca*

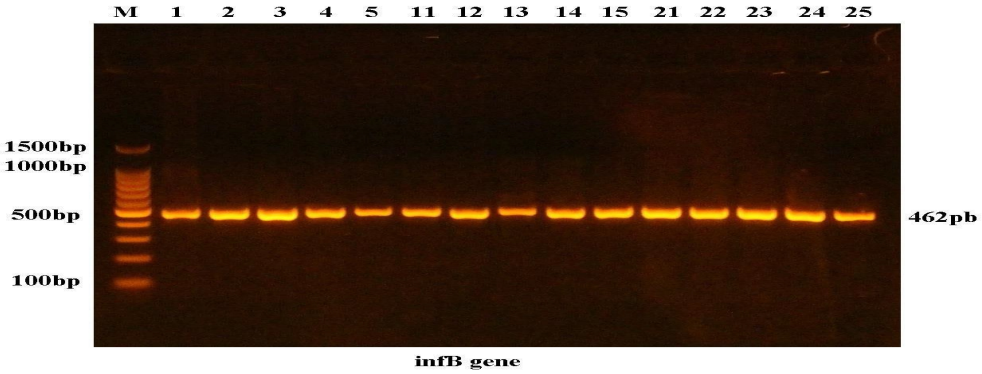
Table (3) morphological characterization, gram staining and biochemical tests results of
K.pneumonia

Genomic DNA Extraction:

DNA was extracted from all bacterial isolates by using rapid bacterial genomic DNA isolation kit (Promega, USA). Extraction results were good for concentration and purity which is determined by using nanodrop spectrophotometer at 260/280 nm. Concentration values of DNA ranged between 200 600 ng/µl while purity
ranged from 1.45-2.1(apendix 2) then subjected to gel electrophoresis in 1.5% agarose, the DNA bands were visible under UV as shown in the figure (2).

Molecular Detection of *InfB* gene in *Klebsiella pneumoniae*:

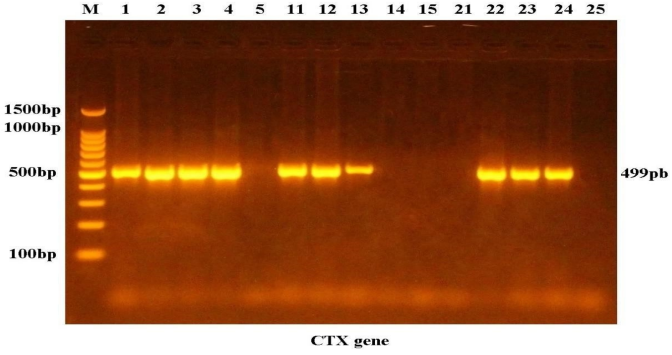
Fig. (2) Showed the result of the polymerase chain reaction (PCR) method to
Detect *Klebsiella pneumoniae* using the *InfB* gene showed that only 50% of The isolates diagnosed by conventional and biochemical methods possess of This gene.



Figure(2) Results of the amplification of infB gene of *Klebsiella pneumoniae* bacterial species were fractionated on 1.5% agarose gelectrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-25 resemble 462bp PCR products

Molecular detection of *CTX* genes *in K. pneumoniae*

The current study showed that 67% of the samples diagnosed by
Molecular methods possessed the *CTX* resistance gene, most of which came
From patients with urinary tract infection, while the isolates that did not
have this gene were from other sources. This result agree with Elif *etal*., (2010) they reported that CTX-M type ESBL were observed in 22.72% of E. coli isolates and frequently found in *Klebsiella pneumoniae* isolates.



Figure(3) Results of the amplification of *CTX gene* of *Klebsiella pneumoniae* bacterial
Species were fractionated on 1.5% agarose gel electrophoresis stainwith Eth.Br. M: 100bp

Ladder marker. Lanes 1-25 resemble 499bp PCR products.

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