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Detecting of Some Klebsiella Pneumoniae Resistance Genes Isolated from Different Sources

Adian Abd Alrazak Dakl[1], Ikram Abbas Aboud Al –Samrraee[2]

Abstract

This research included 45 isolates of Klebsiella pneumoniae from out of 114 specimens gathered from different sources from (animal and human) ; all isolates were diagnosed on Hi-Crome klebsiella Selective agar base and Hi-Crome ESBL Chromagar which isolate just resistant Klebsiella pneumoniae strain of extended spectrum beta lactamase antibiotic group. The discs method (Kirby-Bauer) was used to conduct the sensitivity test for resistant isolates to ascertain Klebsiella pneumoniae's resistance to 24 different antibiotics. . The results investigated that 21 Klebsiella pneumoniae isolates from out of 45 isolates was resistant by growth on ESBL Chromagar and the highest resistance was against Cefuroxime, Cefadroxil, Ceftriaxone,Co-Trimoxazole and Norflxacin, while Klebsiella pneumoniae was less resistant to Cefepime, Cefpirome and Aztreonam and sensitive to Imipenem and Tobramycin,Pipracillin and Etrapenem .

Three Klebsiella pneumoniae isolates was diagnosed molecularly by using conventional polymerase chain reaction, 16S rRNA and detection of some resistance genes (blaOXA48, qnrB, sul1 and aac(6')-Ib-cr). Results showed that when PCR was carried out using the primer that targets the 16S rRNA, all isolates of Klebsiella pneumoniae produced a distinct band with a molecular size of 1250 bp. When using the primer specific for the resistance genes blaOXA48, qnrB, sul1 and aac (6') -Ib-cr, there are two isolates carry three out of four examined resistance genes which gave a band of 744 bp in size for the blaOXA48 gene,469 bp,436bp and 482 bp to qnrB,sul1 and aac(6')-Ib-cr genes respectively. These results suggest that blaOXA48, qnrB, sul1 and aac (6')-Ib-cr genes might be a useful marker to identify resistant Klebsiella pneumoniae strains.

Keywords: *Klebsiella pneumoniae, blaOXA48, multi drug resistance, PCR.*

1. Introduction

Klebsiella is an important pathogenic bacteria in public health because they are animal-borne bacterial pathogens (zoonosis) (1), It has a complex antigenic structure include capsular polysaccharides (CPS) and lipopolysaccharides, which make it is resistant to the several circumstances as environment, action of disinfectants as well as many antibiotics that explain the lethal effects of Klebsiella . In addition to possess other virulence factor such as adhesions, iron acquisition systems and production of endotoxin(2).

These microorganisms in human can cause primary pneumonia, acute intestinal infections, It is the second bacteria , after E. coli that causes urinary tract infection (3) , meningitis and nosocomial infections are common and remain a major cause of mortality and morbidity worldwide (4). While in animals, Klebsiella were isolated from respiratory diseases from various domestics including sheep (5) and can cause sepsis in lambs(6)

[1] Department of Biology, College of science, Al Muthanna University, Iraq.
[2] Department of Microbiology, College of Veterinary Medicine, Baghdad University, Iraq.

moreover, is an important pulmonary bacteria for calves, foals, horses, camels and sow It is associated with many animal ailments and a variety of systemic infections and diarrhoea in calves , mastitis in cattle, metritis in mares and meningitis in piglets (7) . Ibrahim(2008) investigates the pathological alterations brought on by Klebsiella pneumoniae infection in the urinary organs (8).

Althought Klebsiella pneumoniae is known to its virulence also escapes from the host defense by changing its lipid A structure from the powerfully antagonistic hexa-acylated form to the weakly antagonistic hepta-acylated form to stop the rapid and persistent immune activation.(9).

and distinguish with ability to antibiotic resistance rapidly. It revealed multidrug resistance by different types of mechanisms, the manufacturing of enzymes that render antibiotics inactive, including carbapenemases (KPC), amp C beta-lactamase, and New Delhi metallo-lactamase-1 (NDM-1), is one of the best known mechanisms of resistance (10). Quinolone resistance mechanism has also been reported in Klebsiella pneumoniae, including enzymatic mutations in DNA gyrase, topoisomerase IV and active efflux of fluoroquinolones (11). Animals mostly use antibiotics as growth boosters,prophylactic and therapeutic agents resulting in prevalence of antibiotic resistance not only in animals but also in human(12).

This work aimed to investigate the attendance of some resistance genes (blaOXA48, qnrB, sul1 and aac(6')-Ib-cr) in clinical isolates of K. pneumoniae isolated from different sources (human and animal).

2. Materials and Method

2.1. Samples

In total,114 clinical specimens were collected from different sources ,64 animal swab specimen (32 nasal and 32 rectal swab) also 50 human patients with urinary tract infections (15 and 35males and females) respectively from Al-Hussien Teaching hospital in Al-Muthanna ,Iraq, for the period from Mars 2022 to June 2022 .

2.2.Isolation and Identification:

Samples were isolated and diagnosed using MacConkey agar,Hi-Crome ESBL agar base and Hi-Crome klebsiella Selective agar base culture media. Vitek 2 system was utilized for isolation diagnosis (13) ,and confirmed by the polymerase chain reaction (PCR) targeting the 16S rRNA gene (14)

2.3.Antibiotic Sensitivity Test

The discs diffusion method (Kirby- Bauer) was performed to test the bacterial isolates sensitivity and determine Klebsiella pneumoniae's resistance to 24 different antibiotics as three groups(OD272,OD285,OD296) each group contain eight antibiotics participate together with ring shape .

In addition to use Etrapenem as single antibiotic to examine suspected isolates, the measurement of the inhibition zone diameter with around the antibiotic discs was performed and compared with the tables of international measurements (15). Klebsiella pneumoniae was considered MDR (multi drug resistance) when it was impervious to at least three different types of antibiotics (16,17).

2.4.Polymerase Chain Reaction

DNA extracted of bacterial isolates was performed by the FavorPrep Blood/ Cultured Cells Genomic DNA Extraction Mini Kit. Polymerase chain reaction (PCR) test was done with 16S rRNA gene with primer sets, forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' , and reverse primer 5'-

GGTTACCTTGTTACGACTT- 3' then detection the antimicrobial resistant genes by the amplification of the Klbsiella pneumoniae target genes (blaOXA48, qnrB, sul1 and aac (6')-Ib-cr). The reaction was carried out in a 25 microliter volume ,5 microliter of a ready Master Mix ,1 microliter of each primer , and 1.5 microliter of DNA, while 16.5 microliter nuclease-free water was used to complete the volume. The PCR program of DNA amplification as indicating in table-1(18,19).

Table (1): PCR amplification program for KPC primer (18,19)

Stage	Temperature (time)	N umber of cycle
Initial denaturation	95^0C for (3) min	1
Denaturation	95^0C for (30) sec	30
Annealing	55^0C for (30)sec	
Extension	72^0C for (1)min	
Final extension	72^0C for (10) min	1

2.6. The resistance genes of Klebsiella pneumoniae

Forward and Reverse primers (Prmega, USA) for the detection of blaOXA48, qnrB, sul1 and aac(6) -Ib-cr genes were chosen according to Ballén et al (20) . These primers came in lyophilized form, were dissolved in sterile deionized distilled water to give a final concentration of 100 pmol/l as stock solution, and were stored at -20 to prepare a 10 pmol/l concentration as work primer suspended, with 10 ml of the stock solution in 90 ml of the deionized distilled water to get a final volume of 100 l. Table 2 contains a list of the primer sequences that were utilised in this study.

Table (2): The sequence of primers that used in this research

Primer	**Sequence**	**Primer sequence 5`---3`**	**Size of product (bp)**
blaOXA48	F	TTGGTGGCATCGATTATCGG	744
	R	GAGCACTTCTTTTGTGATGGC	
qnrB	F	GATCGTGAAAGCCAGAAAGG	469
	R	ACGATGCCTGGTAGTTGTCC	
sul1	F	CTTCGATGAGAGCCGGCGGC	436
	R	GCAAGGCGGAAACCCGCGCC	
aac(6')-Ib- cr	F	TTGCGATGCTCTATGAGTGGCTA	482
	R	CTCGAATGCCTGGCGTGTTT	

3. Results

The prevalence of Klebsiella pneumoniae among clinical specimens was 45 (31.2%) isolates distributed among 37 animal and 8 human isolates .the highest percentage of the isolates was obtained from animal rectal swabs at 12% . while the human isolates were 7% .

3.1. Antibiotic Susceptibility test

Klebsiella pneumoniae isolates were reported high resistance towards various kinds of antibiotics Cefuroxime, Cefadroxil, Ceftriaxone,Co-Trimoxazole, Norflxacin and Aztreonam , while Klebsiella Pneumoniae revealed less resistant to Cefepime, Cefpirome. In contrast, Klebsiella pneumoniae showed sensitive to Imipenem and Tobramycin,Pipracillin and Etrapenem

3.2. Identification of Klebsiella pneumoniae by PCR :

The DNA extraction from three K. pneumoniae isolates was made by the Favor prep blood/ cultured cells genomic DNA extraction mini kit. The purity and the concentration of DNA were ranged from (45-95-97) ng/ul, respectively. All three isolates in this study showed PCR product with 1250 bp by Klebsiella pneumoniae according to the amplified piece of the 16S rRNA gene that performed Klebsiella pneumoniae (21) (Figure 1)

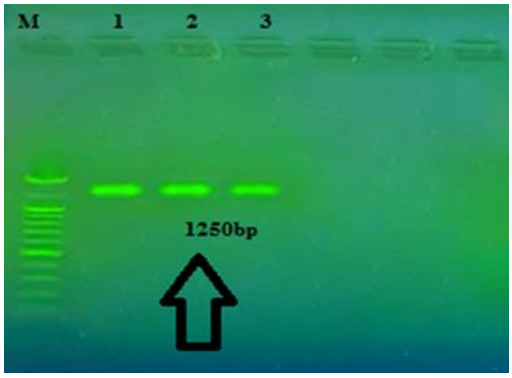


Figure 1) PCR product the 16S)

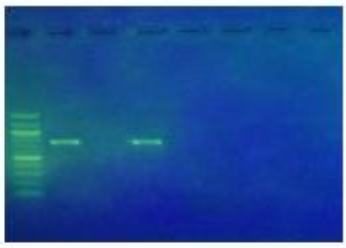
Detection of resistance genes in Klebsiella pneumoniae isolates:

PCR were carried out to three Klebsiella pneumoniae isolates, two isolates gave specific identification for blaOXA48 and qnrB genes while one isolate gave positive result of Sul1 gene and the other isolate identified aac(6')-Ib- cr (Table :3)

Table (3): Identification of resistance genes in Klebsiella pneumoniae isolates

No. isolate	PCR Product of 16SrRNA	blaOXA48	qnrB	aac(6')-Ib-cr	sul
1	+	+	+	-	+
2	+	-	-	-	-
3	+	+	+	+	-

(Figure 2) blaOXA48



(Figure 3) qnrB

(Figure 4) Sull

(Figure 5) aac(6')-Ib-cr

The prevalence of Klebsiella pneumoniae was 31.2% in this study, this result was supported by study performed by Alyassari et al and Nirwati et al., 2019 (22,23,24).

In contrast other studies reported different percentages such as (4.03%,7.6%) and 17.36% (25,26,27).

These variations in the mean prevalence rates across different research may be due to variations in the population's hygiene behaviors and geographic location (28)

The highest percentage of Klebsiella pneumoniae isolates were reported among rectal swap animals since the highest number of collected samples in this study were from animals; moreover, the time collect of samples between March to June help to increase prevalence percentage rate of Klebsiella pneumoniae infection.

Cefuroxime, Cefadroxil, Ceftriaxone,Co-Trimoxazole and Norflxacin showed the lowest effect towards K. pneumoniae isolates, while Imipenem ,Tobramycin ,Pipracillin and Etrapenem . revealed the highest effect. These results were supported by studies performed by (29,30).

Many studies have found that the incidence rate of bacterial resistance varies depending on the population's exposure to antimicrobial treatments, their hygienic culture, and the type of clinical samples that were analyzed (31).

The use of antibiotics in the community, hospitals, the environment, agriculture, and animal production are just a few of the variables that contribute to the spread of antibiotic resistance. Additionally, since it is possible to purchase antimicrobials without a prescription (32).

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