Detection of Plasmid Profile coding Antibiotic Resistance of *Klebsiella spp*. Isolated From Different Infection

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Abstract

One hundred and fifty five of Gram-negative-lactose fermented bacteria grown on MacConkey agar were collected from burns, wounds, UTI and RTI in two hospitals of Najaf province. The isolates were identified according to cultural characteristics and biochemical activities. 43 (27.7%) isolates were *Klebsiella* spp. represented by 35 (81.3%) were *K. pneumoniae* subsp. *aerogenes*, 4 (9.3%) was *Klebsiella pneumoniae* subsp. *pneumoniae*, 2 (4.6%) was *Klebsiella oxytoca*, as well as one isolate for each of *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *Rhinoscleromatis*. Plasmid profiles of all Klebsielae isolates were studied and the results have revealed that 37 (86%) isolates had two distinct plasmid bands.

Itroduction :

Klebsiella is the oldest genus known among the *Enterobacteriaceae* family; the normal habitat of this bacteria is the intestinal tract of human and animal, but may be transferred to another site causing a wide range of infections, such as burns, wounds, respiratory tract and urinary tract infections; these infections become difficult to be treated because of the increased ability of Klebsiellae to resist different types of antibiotics (Fluit *et al*., 2001). The epidemiology of nosocomial outbreaks can be more complex when the resistance is mediated by several mechanisms,the important one of which is the production of enzymes encoded by several genes that are carried on some bacterial plasmids, β-lactamase and extended spectrum β-lactamase. Extended spectrum β-lactamases are mostly plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactam antibiotics, including different types of penicillins and cephalosporines (Lautenbach *et al*., 2004) . The isolates-producing ESBL are usually predominant in hospitals infetions where the antibiotics are used frequently and the patient`s condition is critical, the prevalence of ESBLs in the *Enterobacteriaceae* was up to 37.5% in nosocomial isolates, versus 6% in community bacterial isolates (Lautenbach *et al*., 2004).The spread of plasmid-encoded β-lactamase enzymes occurs with concomitant transmission of epidemic isolates directly from patient to patient (Pagani *et al*., 2000). *Klebsiella pneumoniae* contain many plasmids that differ in numbers and molecular weight, carrying different types of genes including those encoding extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, inhibitor resistant *TEM* β-lactamases and metallo β-lactamase enzymes. These enzymes confer resistance to various antimicrobial agents including the third and fourth generation cephalosporins, cephamycins, monobactam β-lactamase, β-lactamase/inhibitor combinations and carbapenems (Essack *et al*., 2004). Subha *et al*. (2002) revealed that *K. pneumoniae* contains a common large plasmid (89 kb) which confer resistance to ampicillin, kanamycin and chloramphenicol and intermediately resistant to amikacin. Furthermore, Quale *et al*. (2002) found that the transfer of plasmids is influenced by environmental conditions thus the experiments methods in vitro showed transfer of 108 and 157 kb plasmid, whereas the experimental conjugative methods recorded transfer of the 89 kb plasmid. Moreover,

in vivo the experiments illustrated that the transfer of the 108 kb plasmid was observed in the colonizing experiment where the plasmid transferred to the indigenous *E. coli*, plasmids from the clinical *K.pneumoniae* isolates appear to be highly conjugative plasmids, thus, both the plasmids (89 and 108 kb) were transferred at high frequency (Subha *et al*,. 2002).

The methods :

Specimens collection:

155 isolates of gram negative bacterial grown on MacConky agar were collected from two hospital in Al-Najaf province ,129 isolates from Al –Sader hospital and 29 isolates from Al-Zahraa hospital The isolates where represented by 64 isolates from burin infections ,45,25isolates from urinary and respiratory tract infections respectivlly and 21 isolates from wounds infections .

-Identification of bacterial isolates:

The following criteria were dependent to identification of all bacterial isolates that including the following below:

--Morphological and cultural characters (Macfaddin, 2000).

--Biochemical activities including IMViC test ,TSI reaction ,motility test and urease test(Macfeddin ,2ooo).

- DNA Extraction and Purification

The method of Popiech and Neuman, (1995) was used for extraction and purification of bacterial DNA with some modifications as followes:

1. Bacterial cells of 5 ml of fresh broth culture where precipitated by centrifugation at 5000 rpm for 10 minutes.

2. The bacterial cells were rewashed many times (2-3) with TE buffer (TE buffer was prepared by dissolving 0.05 M Tris-OH and 0.001 M of EDTA in 800 ml distilled water, the PH was adjusted to 8 and the volume was completed to one liter with distilled water, then autoclaved at 121 C° for 15 minutes and stored at 4 C° until used).

3.Then the pellet was resuspended in 5 ml SET buffer, (SET buffer was prepared by dissolving 20 mM Tris-OH ,25mM of EDTA and 75mM NaCl in 750 ml distilled water, PH was adjusted to 8 and completed with distilled water to one liter, then autoclaved at 121 C° for 15 minutes, and stored at 4°C until used).

4. Six hundred µl of fresh 25% SDS was added, and 2 ml of 5. M NaCl solution was added to the lysate and mixed by inversion, the solution was incubated for 5 minutes at 55 C°, then, mixed thoroughly by inversion, and let to be cooled to 37 C°.

5. Five ml of phenol: chloroform: isoamylalcohol (25:24:1 v/v) was added to the lysate and mixed by inversion for 15-30 minutes at 25 C°, it was spun by centrifuge 6000 rpm at 20 C° for 10 minutes.

6.Then aqueous phase which contains nucleic acid was transferred to fresh tube, Isopropanol (0.6 volume) was added to extract and mixed, after 3 minutes DNA aspirated by micropipette.

7. The extract of DNA has been washed with 70% ethanol, left to air drying, then dissolved in 100-250 µl TE buffer, pure DNA extract was stored at -20 C° until use.

--Result and Discussion :

-Bacterial distribution : The *Klebsiella* spp. clinical isolates have been detected in 43 (27.7%) isolates of the total number of clinical Gram-negative isolates (155) ,according to criteria were listed in table-1. The isolates obtained from different infections were represented by 17 (39.5%) isolates from burns infections, 14 (32.5%), 5 (11.8%) and 7 (16.2%) isolates from UTI, wounds and RTI infections respectively (Table -2). Twenty nine of bacterial isolates have been recovered from infected hospitalized patients represented by 17 isolates from burn infections, 7 isolates from wounds infections, 3 isolates from respiratory tract infections, and 2 isolates from urinary tract infections. The results versus 14 isolates were recovered from out patients(12 UTI and 2 RTI), our findig may be true, because Klebsiellae is one of the most important opportunistic pathogens commonly predominant in hospital environment, particularly in medical and surgical instruments like catheters in addition to bed of patients in burns and surgical intensive care unit which are critical source of Klebsiellae nosocomial infections (Fang *et al*., 2005). The results revealed that *K.pneumoniae* subspecies *aerogenes* was the most common member of *Klebsiella* spp. causing different infections, it was identified in 35 (81.4%) isolates from the total number of *Klebsiella* spp. isolates, (Table -3). This result is in agreement with many studies established by Kim *et al*., (2002)and Iroha *et al*., (2009) which have concluded that *K.pneumoniae* subspecies *aerogenes* was the most common member of *Klebsiella* spp., *K.pneumoniae* subsp. *pneumoniae* which was recorded in 4 (9.4%) isolates, whereas *K.pneumoniae* subsp. *ozanae* and subsp. *rhinoscleromatis* were identified in 1(2.3%) for each one. *K.oxytoca* was recorded in 2(4.6%) isolates from the total number of *Klebsiella* spp. All Klebsiellae clinical isolates has been tested in order to detect the plasmid pattern of each isolate, the results have clarified that 37 of 43 Klebsiellae isolates (86%) carry two distinct plasmids, in contrast, only 6 isolates (14%) appear to be having no plasmids (Figure -1). Moreover, these isolates were highly resistant to common antibiotics indicating that the resistance of these isolates may be encoded by chromosomal genes; the results of our study indicated that there is no relation between the number of plasmids and ability of antibiotic resistance.

Gori *et al*. (1996) reported that the epidemiology of nosocomial outbreaks can be more complex when the spread of plasmid-encoded β-lactamase enzymes occurs concomitantly transmission of epidemic isolates directly from patient to patient. However, *Klebsiella pneumoniae* isolates contain many plasmids that differ in numbers and molecular weight, carrying different types of genes including those encoding extended-spectrum β-lactamases (ESBLs). The number of resistance genes carried on the plasmids of multiresistant *K. pneumoniae* is usually more than one, and occasionally as many as five genes were reported (Poirel *et al*., 2004). In fact, coexistence of broad-spectrum β-lactamases with ESBLs, ESBLs with *AmpC* β-lactamase, ESBLs or multiple ESBLs with metallo-β-lactamase has become common in multi-resistant *K. pneumoniae* isolates (Yan *et al*., 2001). Extended spectrum β-

lactamases were the most prevalent in *K. pneumoniae*, mostly encoded on large plasmids with size between 80 to 160 kbp (Hanson *et al*., 2005).

The study of Woodford *et al*., (2008) pointed out that *Klebsiella pneumoniae* contains two plasmids of molecular weight approximately 95 kbp and 200 kbp size encoding beta-lactamase enzymes represented by *blaTEM-1, blaSHV-12, blaCTX-M-3* and *blaDHA-1*. Quale *et al*. (2002) concluded that random administration of third generation cephalosporins or aminoglycosides is a risk factor that enhances the acquisition of plasmid-encoded ESBLs. *K. pneumoniae* have the ability to transfer plasmids containing resistance genes to an indigenous *E.coli* during therapy period (Essack *et al*., 2004). Ensor *et al*. (2006) revealed in his study that *K. pneumoniae* contains a common large plasmid 89 kbp which confer resistance to ampicillin, kanamycin , chloramphenicol and amikacin. Furthermore, the same study found that the transfer of plasmids is influenced by environmental conditions; thus, the experiments methods in vitro appeared transfer of 108 and 157 kbp plasmid, whereas the experimental conjugative methods showed transfer of the 89 kb plasmid. (Granier *et al*., 2002).

Table- 1 Biochemical test of bacterial identification

Bacteria / Test	*Klebsiella pneumoniae* subspecies				*K..oxytoca*	*E.coli*	*Enterobacter*
	aerogenes	*ozaenae*	*Pneumoniae*	*rhinoscleromatis*			
Motility	-	-	-	-	-	+	++
MR	-	+	+	+	V	+	v
VP	+	-	-	-	V	-	+
Citrate	+	V	+	-	+	-	+
urease	+	+	+	-	+	-	v
TSI	A/A	A/A	A/A	ALK/A	A/A	A/A	A/A
Indole	-	-	-	-	+	+	-

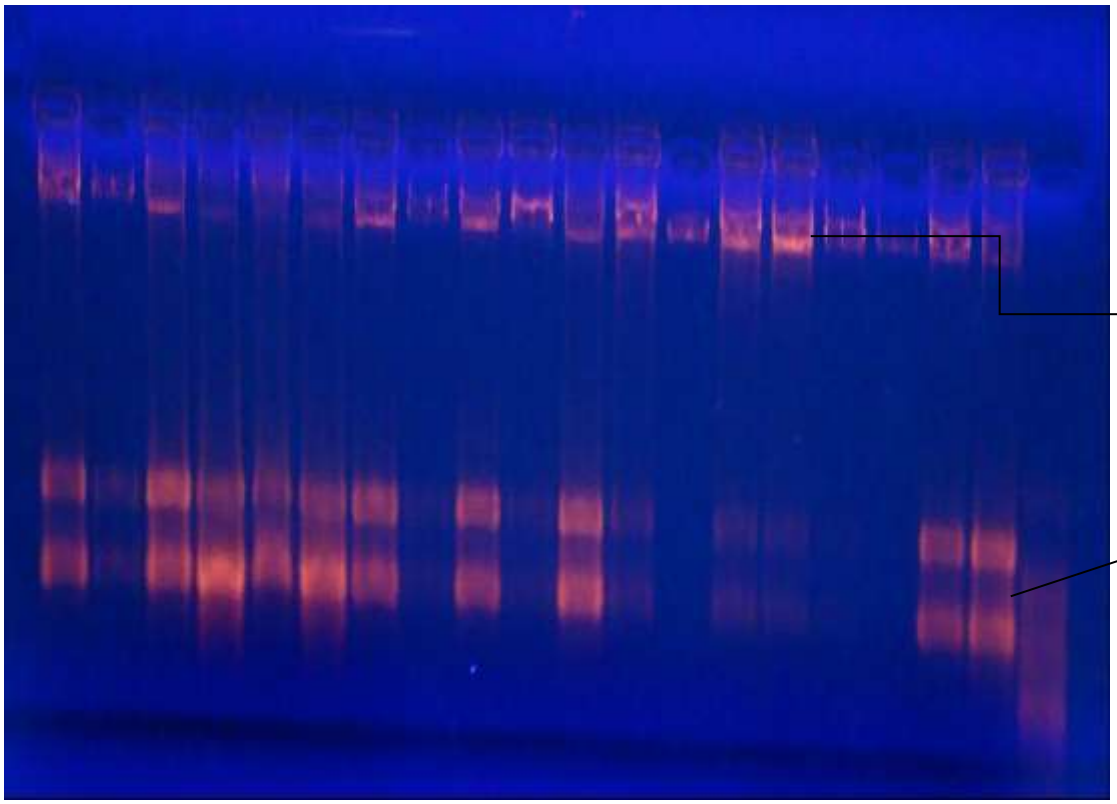
Table -2 Distribution of Klebsiellae isolates according to hospital:

Patients \ Infection site		Burns	Wounds	UTI	RTI	Total		Percentage
Outpatients	Alsader	_	_	8	1	9	14	32.6%
	Alzahra	_	_	4	1	5		
Hospitalized patients	Alsader	17	7	1	3	28	29	67.4%
	Alzahra	_	_	1	_	1		
Total (%)		17 (39.5%)	7 (16.2%)	14 (32.5%)	5 (11.8%)	43		

Table -3 Distribution of *Klebsiella* spp. According to Infection Sites:

Bacteria \ site of	*K.pneumoniae* subspecies				*K.oxytoca*	Total
	aerogenes	*pneumoniae*	*Ozaenae*	*rhinoscleromatis*		
Burns	15	1	1	_	_	17
Wounds	6	1	_	_	_	7
UTI	12	1	_	1	_	14
RTI	2	1	_	_	2	5
Total	35 81.5%	4 9.4%	1 2.3%	1 2.3%	2 4.6%	43

Figar -1 Plasmid profile of *Klibseilla spp* isolates



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