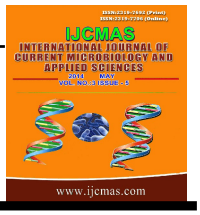
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 5 (2014) pp. 26-31**
http://www.ijcmas.com



Original Research Article

Molecular characterization of *aerobactin* gene among *Klebsiella* isolated from Wound and Burn Infections

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Keywords

Klebsiella, capsule, aerobactin, gene, PCR, siderophore

A B S T R A C T

Hundred and twenty five Gram-negative-lactose fermented bacteria grown on MacConkey agar were collected from burn and wound infections, in Al-sadar hospital, An Najaf, Iraq. The bacterial isolates were identified according to cultural characteristics, biochemical test and API20E, the results revealed that 40(32%) isolates were identified as *Klebsiella spp*; all of them 40(100%) isolates were belong to *K. pneumonia*. *Klebsiella* isolates that producing siderophore were tested and found totally produced siderophore. Furthermore these strains subjected to molecular studies by PCR technique focusing on aerobactin gene. The results pointed out that 35 (87.5%) of bacterial isolates have *aerobactin* genes coding for producing siderophore. In the present study we found *Klebsiella pneumonia* totally produce siderphores were expressed *aerobactin* genes.

Introduction

Klebsiella is gram negative rod in shape. It is the important member of the Enterobacteriaceae family; the normal habitat of this bacteria is the intestinal tract of human and animal, but it was a causative agents causing a wide range of burn, wound, respiratory urinary tract infections and bacteremia. Iron is an essential nutrient for the majority of bacterial species. It plays a key role as a cofactor for the electron transport chain and for various other enzymes (Wieland *et al.*, 2011). However, in aerobic conditions and mammalian tissues, the majority of iron is found as Fe^{3+}; iron entirely sequestered by transferrin and lactoferrin, to grow successfully in host tissues, bacteria must be able to obtain iron from these host transport proteins (Wandersman and Delepelaire, 2004). Different species have evolved a variety of secreted factors in order to obtain iron. Among the bacteria of *Enterobacteriaceae* family, a number of different siderophore systems have developed to fill this role (Wandersman and Delepelaire, 2004). Aerobactin is a hydroxamate siderophore which is produced by a smaller fraction of

enterobacterial strains and has a lower affinity for free Fe^{3+} than either enterobactin or yersiniabactin (Perry *et al.*, 1999). In the genus *Klebsiella*, the production of both enterobactin and aerobactin has been demonstrated. However, enterobactin can be synthesized by all strains (Podschun *et al.*, 2000). Gram-negative isolates grown on MacConkey agar undergo biochemical tests in order to distinguish *Klebsiella* isolates from other members of related lactose fermented bacteria, all biochemical tests (Macfaddin, 2000).

The majority of gene encoding aerobactin has been carried on bacterial plasmids, recent studied revealed that the *aerobactin* gene responsible for produce of siderophore, in addition to molecular detection methods including DNA probes, Oligonucleotide typing and gene sequencing have been used to identify the aerobactin. This study conducted to study *Klebsiella* isolates producing iron chelator related to detect the presence of aerobactin encoding gene

Materials and Methods

Specimens Collection:

Hundred and twenty five Gram-negative, lactose fermented bacteria grown on Macconkey agar were collected from Al-Sadar hospital, AN Najaf, Iraq. The samples were represented by 20 isolates from burn infection and 105 from wound infection, 55 isolates from females and 70 from males. The isolates were transferred immediately to laboratory for culture and identification.

Isolation of Microorganism

The pathogenic *K.pneumonia* were isolated from hospital samples and indentified on the basis of morphological and biochemical characteristics. The final identification of isolate was current out using API 20E and Vatic-2 system.

Siderophore Production Test

M9 medium was inoculated with pure young colonies growing on brain heart infusion agar, incubated in $37C^0$ for 24 h. The appearance of bacterial growth on the surface of medium indicated positive results (Harrigan and MacCancel, 1987).

Isolation of Plasmid DNA and PCR

DNA was isolated from bacterial cultures by phenol: chloroform extraction. The polymerase chain reaction was carried out in final volume of 25µl containing 100 ng DNA, 2 Units of Taq DNA polymerase, 2.5mM MgCl, 10mM dNTP mix and 100pmol of primers for each sample. The DNA amplification was performed using the following conditions: complete denaturation (94°C for 3 min), followed by 35 cycles of amplification (94°C for 30 sec, 32°C for 30 sec and 72°C for 1 min) and the final elongation step (72°C for 2 min). PCR products were separated on 1.5 % Agarose gel.

Results and Discussion

In this study clinical isolate samples were been taken from Alsader hospital at AN Najaf, Iraq. 125 of bacterial colonies isolates were identified as *Klebsiella spp* based on morphological and biochemical characterization. The identification was done based on their morphology, Gram staining, and biochemical test: Indole test, Methyl red test, Vogues – Proskaur test, Citrate test, nitrate reduction, motility test and TSI reaction (Bharti *et al.*, 2008). The results of citrate utilization test, urease test and TSI test, were shown 40 (51.2%), 40

(51.2%) and 78(100%) isolates as *K. pneumonia* respectively. According to final identification with API20E system and Vitec-2 system, the results revealed that the *Klebsiella spp*. have been accounted to be 40 (32%) out of the total number of clinical Gram-negative isolates (125).

The isolates obtained from two infections site were represented by 10 (25%) of isolates was recovered from burn infections specimens and 30 (75%) isolates from wound infection specimens, 20 of bacterial isolates have been obtained from infected hospitalized patients represented by 10 isolates from each of burn and wound infections, versus 20 isolates were recovered from wounds of outpatients.

In this study all bacterial isolates (100%) showed ability to produce siderophore whereas monitored by the growth of bacteria that appeared on the surface of M9 medium as shown in Figure 1.

In PCR amplification results we observed 35 isolates (87.5%) contain aerobactin gene, while 5 (12.5%) of Klebsiella isolates were lack the gene as shown in Figures (2, 3, and 4).

With few exceptions, the results from this study are very encouraging and have demonstrated that the method suitable for use in laboratories. In case of high rate of *K.pneumonia* isolates from clinical samples emphasis on medical problem and may be due to contamination from the environment whereas particularly in medical and surgical instruments, like catheters, bed of patients and in burns and surgical intensive care unit which are critical source of *Klebsiella* nosocomial infections. In addition to colonize of these bacteria to human intestinal as a normal flora, may be get additional source of infection (Fang *et al*., 2005). Our finding can be true, because *Klebsiella* is one of the most important opportunistic pathogens commonly predominant in hospital environment (Fang *et al*., 2005).

Siderophores have been implicated as bacterial virulence factors. The role of iron acquisition systems is basically important in the light of the new findings that siderophores may represent a key front in the interplay between host and pathogen (Flo et al., 2004). In our study, bacterial isolates shown totally production of siderophores and this is because Iron acquisition systems are essential requirement for the growth of pathogenic bacteria (Matthew *et al* ., 2007), it is an essential nutrient for the majority of bacterial species, it plays a key role of electron transport chain and cofactor for various other enzymes (Nelson *et al* .,2005). Furthermore, the iron chelator siderophore enables bacteria to take up the protein-bound iron from and within the host cells (Griffiths *et al*., 1988).

In the present study, *K. pneumoniae isolates* highly expression *aerobactin* gene. However, in this strain, the *aerobactin* receptor gene was located on a small plasmid, transfer of a recombinant plasmid harboring the genes for *aerobactin* and its receptor enhanced the virulence of an otherwise a virulent strain by 100-fold (Nassif and Sansonetti ,1986). In other hands, a phenotype independent of aerobactin production, the mucoid aspect of *K. pneumoniae* colonies, has been associated with the presence of this plasmid. Furthermore, the virulence of K1 and K2 *K.pneumoniae* isolates has been correlated with the presence of a 180-kb plasmid (Podschun *et al*., 2000). In the

Table.1 Primer sequences of rmpA gene

Gene		Primer sequence	Product length
Aerobactin	F	5'-GCATAGGCGGATACGAACAT-3'	556bp
	R	5'-CACAGGGCAATTGCTTACCT-3'	

Table.2 Shown the distribution of *Klebsiellae* isolates according to infection site

	source Infection patient / hospital	Burn	Wound	Total	Percentage
Alsadar Medical City	Outpatients	_	20	20	50%
	Hospitalized patients	10	10	20	50%
Total (%)		10 (25%)	30 (75%)	40 100%	100%

Figure.1 Shown growth of *K. pneumonia* on M9 medium (siderophore production**)**

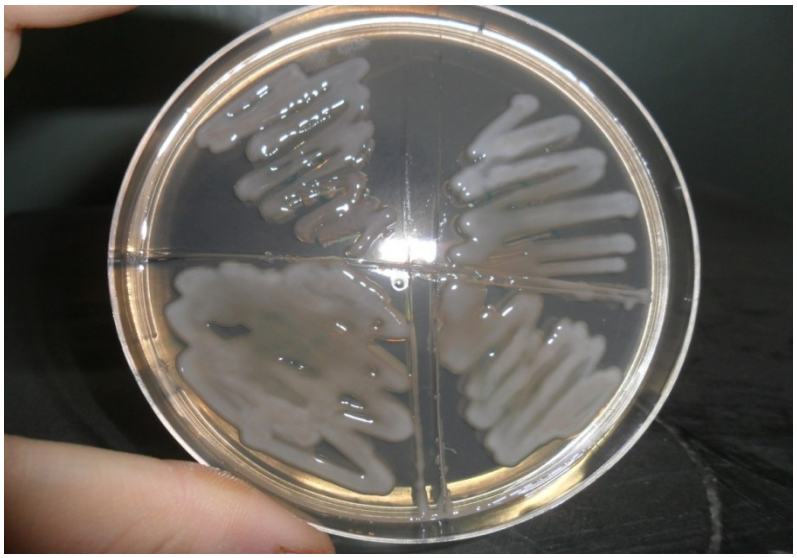


Figure.2 Gel electrophoresis and PCR product of *aerobactin* gene

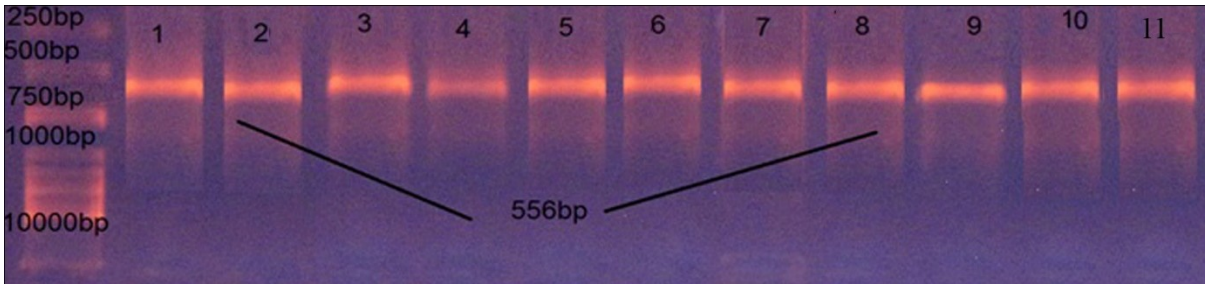


Figure.3 Gel electrophoresis and PCR product of *aerobactin* gene

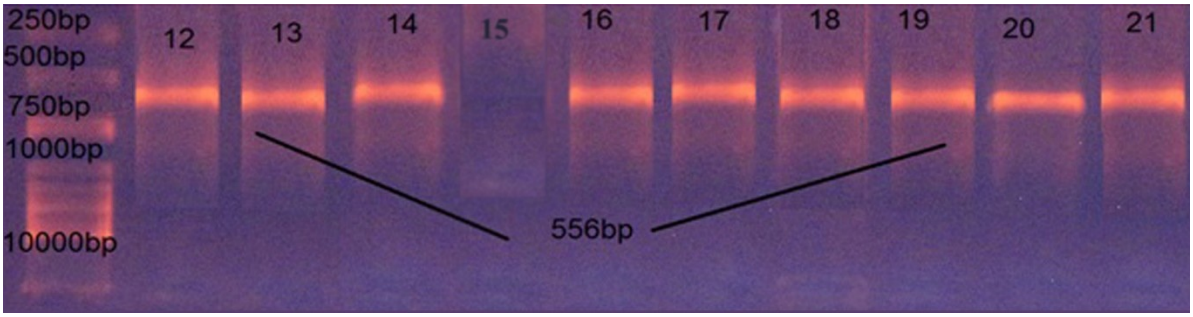


Figure.4 Gel electrophoresis and PCR product of *aerobactin* gene

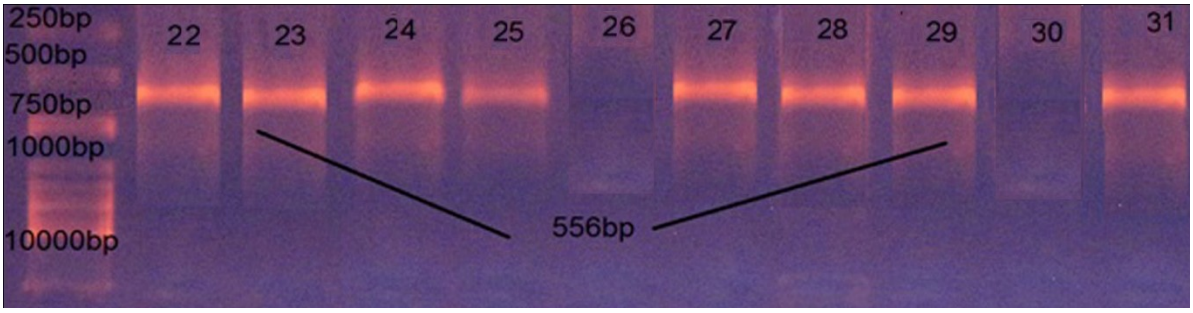
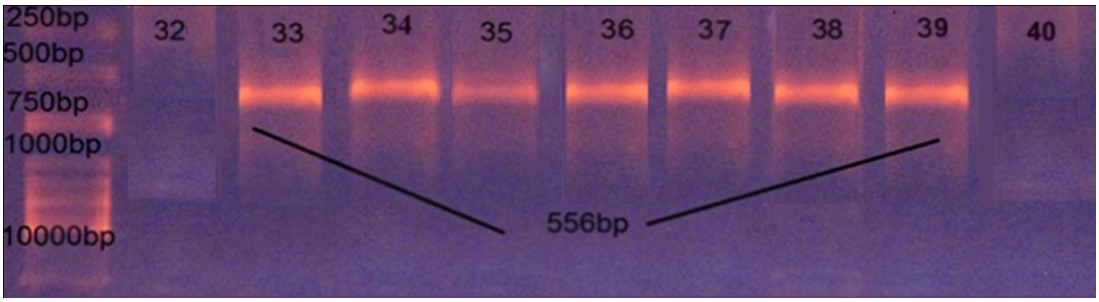


Figure.5 Gel electrophoresis and PCR product of *aerobactin* gene



present study we discussed the correlation between siderphore and *aerobactin* gene whereas *K. pneumonia* strains that produce *aerobactin* were more virulent, but strains not producing this siderophore were less likely to be. Additionally, patients with severe community-acquired infection were more likely to be infected by *aerobactin*-producing strains **(**Gudjon *et al.*, 2009).

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