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PCR detection of *rmpA* gene among *Klebsiella* isolated from wound and burn infections

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ABSTRACT

One hundred and twenty five Gram-negative-lactose fermented bacteria grown on MacConkey agar were collected from burn and, wound infections, in Al- sadar Medical City in Al- Najaf province. The bacterial isolates were identified according to cultural characteristics ,the results revealed that 40(32%) isolates were identified as *Klebsiella spp*, all of them 40(100%) isolates were belong to *K. pneumonia* subsp. *pneumonia* .The capsule of *klebsiella* isolates were tested .the result revealed all *Klebsiella* isolates40(100%) were capsulated ,the gene coding capsule in isolates gave positive results. Genotypically were studied carefully using PCR technique , the results pointed out that 35 (87.5%) of bacterial isolated have *rmpA* gene coding for producing polysaccharide capsule .

INTRODUCTION

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 burn, wound, respiratory and urinary tract infections and bacteremia , *Klebsiella* have capsule that oppose host defenses which depend mainly on impair phagocytosis by polymorphonuclear granulocytes and the bactericidal effect of serum, mediated in large part by complement proteins (Doyle and Evans, 2008) .The majority of gene encoding capsule has been carried on bacterial plasmids, recent studied revealed that. the *rmpA* gene responsible for produce of capsule protect bacteria from macrophage, in addition to molecular detection methods including DNA probes, Oligonucleotide typing and gene sequencing have been used to identify the capsule.

OBJECTIVES OF THE RESEARCH

- To find the reasons for resistance *Klebsiella* to body's defenses
- To prove the presence of capsule gene

MATERIALS AND METHODS

Reagents, Dyes and Culture media including:
Blood Agar Medium , MacConkey Agar Medium, Motility Medium ,Triple Sugar Iron Agar "TSI", Pepton Water Medium ,(MR- VP) Medium, Simmon Citrate Medium , Christenen's Urea Medium

Specimens Collection:

One hundred and twenty five Gram – negative lactose fermented bacteria grown on Macconkey agar were collected from Al sadar Medical City in Al- Najaf province, 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.

Typical characteristics

After an incubation period , the typical characters of *Klebsiella spp.* were used in identification of bacterial isolates; the *Klebsiella* isolates were distinguished by producing large , smooth , elevated and very mucoid colony on blood agar , on Macconkey agar ,they produce large , smooth , pink color (lactose fermented) with elevated and mucoid colony (Bharti *et al.*, 2008).

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Biochemical tests

Carried out the following tests**:**

Indole test ,Methyl red test ,Voges –Proskaur test, Citrate test

Investigation of Capsule

The negative staining of Indian ink was Inquiring to detect the presence of bacterial capsule, according to Atlas (1995) :

- Mixed one colony of young culture with a drop of normal saline on clean slide. A drop of Indian ink was added and mixed with loop carefully , then spread the mixing by edge of anther slide (angle 45 degree). The slide was examined directly under the oil lens, the formation of clear zone around bacterial cell indicated the production of capsule .

plasmid DNA Extraction and Purification

It has been prepared according to recommendation company product (promegia)

PCR Amplification

The method described by (Joshi and Albert, 2009) was used to perform PCR amplification. The oligonucleotide PCR primers specific for the capsule(table-1).

Table –1 primer sequence of *rmpA* gene

Gene	Primer sequence	Product length	reference
RmpA	F 5'-ACTGGGCTACCTCTGCTTCA-3' R 5'-CTTGCATGAGCCATCTTTCA-3'	535pb	Nassif *et al.*,1989

RESULTS AND DISCUSSION

Culture and Identification of Bacterial Isolates

The isolates were cultured on suitable media, including blood agar and MacConkey agar, and incubated at 37C° for 18-24 hour. After incubation period, the identification of bacterial isolates was performed depending on the following criteria:

Morphological and Cultural Characteristics

The characters of the bacterial colonies grown on MacConkey agar were studied; the lactose fermenter (pink color) and mucoid colonies have been taken in consideration, since *Klebsiella spp.* typically produced large, rounded, mucoid (due to thick polysaccharide capsule) and pink color colonies on MacConkey agar , while on blood agar *Klebsiellae* produce smooth, convex, rounded and usually non-hemolytic colonies

Biochemical Tests

All Gram-negative isolates were grown on MacConkey agar undergo biochemical tests in order to distinguish *Klebsiella* isolates from other members of related lactose fermented bacteria , all biochemical tests have been carried out according to MacFaddin (2000). *Klebsiella spp*. is simply differentiated, because all members of *Klbsiella spp*. appear non-motile .

78 (62.4%) of isolates has given negative results for oxidase test , all members of *Klebsiella spp* are negative for oxidase test. 38 (48.7%) isolates give positive results for indole test, while 40(51.3%) isolates gave negative results, all members of *Klebsiella spp* are negative for indole test the break down tryptophan for nutritional lead to released indole that can be detected through the use of Kovacs' reagent, which is reacts with indole and produces a red color on the surface of peptone water .(Macfaddin,2000)

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The results have been indicated that 78(100%) isolates were positive for methyl red test, *all Klebsiella pneumoniae* subspecies *pneumoniae* that gave positive result ,fermentation of bacteria to glucose which lead to accumulation of the acid in the MR-VP medium causing decrease of pH, lead to formation red colour when methyl red reagent was added, (Bharti *et al*., 2008).

The positive results for citrate utilization test were detected in 40 (51.2%) isolates, the *K. pneumonia* subsp *pneumonia* give positive result , indicating from, change the color of Simmon's citrate slants to blue with bacterial growth as a results from used the citrate as a carbon source .40 (51.2%)of bacterial isolates were positive for urease test *K. pneumonia* subspecies *pneumonia* give positive results due to the ability of these microorganisms to produce urease enzyme which breakdown urea and release ammonia(NH3), which in turn lead to increase pH of the medium, causing change the indicator of medium to pink color (MacFaddin, 2000).

On triple sugar iron agar (TSI) 78(100%) isolates gave Acid/Acid result without gas. *K. pneumonia* subsp *pneumonia* have able to fermented different types of sugars produces large amount of acid in the test tube, lead to change phenol red indicator to yellow but without producing gas.

***Klebsiella spp.* Clinical Isolates**

The *Klebsiella spp*. clinical isolates 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%) isolates from burn infections, 30 (75%)isolates from wound infection, (Table -3). 20 of bacterial isolates have been recovered from infected hospitalized patients represented by 10 isolates from burn infections, The versus 20 isolates were recovered from wounds of outpatients, the finding may be true, because *Klebsiella* is one of the most important opportunistic pathogens commonly predominant in hospital environment(Fang *et al*., 2005).

Table -2 distribution of *Klebsiellae* isolates according to infection site:

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

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 this bacteria to human intestinal as a normal flora, may be get additional source of infection (Fang *et al*., 2005).

Phenotypic detection of capsule

All isolates that produce mucoid growth on solid media were examined to inquiring of capsule, using negative staining of Indian ink. The result of direct examination appear that all bacterial isolates (40) have capsule (100%) .

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4.4 .2.1 PCR Detection of *rmpA* Gene

The results of virulence factors genes detection clarify that 35 isolates (87.5%) of capsule producers isolates carrying *rmpA* gene while 5 (12.5%) of *Klebsiella* isolates were lack the gene. This results were closely related to results of Hansen *et al* .,(2007), they found that 93% of *klebsiella pneumonia* carrying *rmpA* gene. Hypermucoviscous capsule, a protective layer surrounding the cell surface, is important factor for pathogenicity of *Klebsiella pneumonia.* The public health relevance of this research is to understand a unique regulator that controls hypermucoviscous capsule formation and composition(Valley ,2011)

This gene should be considered a component of K1 capsule formation, mutation in *rmpA gene* lowers in 13%–29% of capsule material (Yeh *et al*., 2006) Chromosomal *magA* gene, which encodes a structural outer membrane protein essential for the production of the exopolysaccharide web, is associated with hypermucoviscous phenotype (Chuang *et al*., 2006). Study from two largest medical centers in southern Taiwan from (July 2003 to December 2004). demonstrated that of nosocomial strains causing liver abscesses and community-acquired *K. pneumoniae* strains are positive for *rmpA*, but not for *magA*, are significantly associated with the virulent hypermucoviscosity phenotype and purulent tissue infections in the liver and other organs. (Yeh *et al*., 2006)

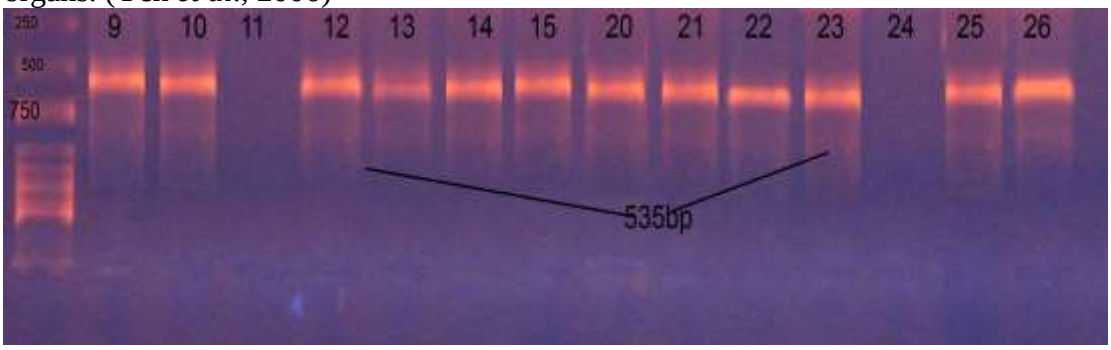

Figure *rmpA* gene of *klebsiella pneumonia*

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الخلاصة

تم عزل مائة وخمسة وعشرون من البكتيريا السالبة لصبغة جرام المخمرة للاكتوز المزروعة على أجار ماكونكي والمعزولة من إصابات الحروق والجروح من مدينة الصدر الطبية في محافظة النجف، خلال 2010/12/10-2010/9/10. تم التعرف على العزلات البكتيرية وفقا لخصائص الصفات الزرعية والكيمياء الحيوية بالإضافة إلى استخدام عدة Api 20E. وقد كشفت النتائج إن 40 (32٪) البكتيريا المعزولة هي *klebsiella spp*.

وان هذه العزلات40 (100٪) تنتمي إلى *K .pneumonia subsp pneumonia*. وقد أجريت اختبارات لتشخيص عوامل الضراوة في عزلات *Klebsiella*، وذلك باستخدام أساليب مختلفة. وأظهرت النتائج أن جميع عزلات *Klebsiella* 40(100%)تحتوي على كبسولوراثيا، تمت دراسة جينات العزلات التي أعطت نتائج إيجابية باستخدام اختبار تفاعل البلمرة التسلسلي تقنية (PCR). وأشارت النتائج(87,5%) من البكتريا المعزولة لها rmpA الجينات المسؤولة عن إنتاج كبسولة السكاريد.

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