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 isolates 40(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).
Biochemical tests
Carried out the following tests:
- Indole test
- Methyl red test
- Voges–Proskauer 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 another 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>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Product length</th>
<th>reference</th>
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<tbody>
<tr>
<td>RmpA</td>
<td>F 5’- ACTGGGCTACCTCTGCTTCA-3’</td>
<td>535pb</td>
<td>Nassif et al., 1989</td>
</tr>
<tr>
<td>RmpA</td>
<td>R 5’- CTTGCATGAGCCATCTTTCA-3’</td>
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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 37°C 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 Klebsiella 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)
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* sub species *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>
<thead>
<tr>
<th>Table -2 distribution of <em>Klebsiella</em>e isolates according to infection site:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>source Infection patient</strong></td>
</tr>
<tr>
<td>Alsadar Medical City Hospital Outpatients</td>
</tr>
<tr>
<td>Alsadar Medical City Hospitalized patients</td>
</tr>
<tr>
<td>Total (%)</td>
</tr>
</tbody>
</table>

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%).
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

REFERENCES


Valley.(2011) rmpA a Unigue Virulence Regulator in Emerging *Klebsiella pneumonia*. J. Infectious Diseases. p 10-069


**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 01/9/2010-01/00/01. 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 *Klebsiella spp* هي *Klebsiella subsp pneumonia*.

وقد أجريت اختبارات لتشخيص عوامل الضراوة في عزلات *K. pneumoniae subsp pneumonia* 40 (100%) تنتتمي إلى *Klebsiella*، وذلك باستخدام أساليب مختلفة. وأظهرت النتائج أن جميع العزلات تحتوي على كبسولوراثيا، تمت دراسة جينات العزلات التي أعطت نتائج إيجابية باستخدام اختبار تقنية (PCR) 7,5% من البكتيريا المعزولة لديها الجينات المسؤولة عن إنتاج كبسولوراثيا السكاريد.