Study Some Virulence Factors Of *Klebsiella pneumoniae* Isolated From Clinical Sources

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Abstract:

This study was carried out in General Al-Nasseryia Hospital ,at Al-Nasseryia city. 99 isolates of *Klebsiella pneumoniae* (61 isolates from surgical wound infections and 38 from hospital environment ) were determind the some virulence factors .

All isolates of *Klebsiella pneumoniae* were having capsule (100%), 98% having type I pili or mannose sensitive hemagglutination (MSHA), 100% having type 3 pili or mannose resistant *Klebsiella* hemagglutination (MR/K-HA) and 99% of them producing siderophores.

Introduction:

*Klebsiella pneumoniae* is present in the respiratory tract and feces of about 5% of normal individuals , it is an important cause of hospital- acquired infection (1).

Stock and Wiedemann (2) reported that *K. pneumoniae* are opportunistic pathogens that cause a wide rang of infection in man .

For instance in an study of Aboott (3) found intraoperatively , *Kebseilla spp* had a 68% probability of causing a surgical wound infections ,approximately one third of patients carry *Klebsiella spp* in their stool, but carriage rates may increase as much as three- fold with hospitalization and antimicrobial usage in adults.

Podschun and Ullmann (4) reported that the surgical wound infections due to *Klebsiella* were associated with a server pyogenic infection which has fatality rate if untreated , the emergence of *Klebsiella* as an important to the use of antibiotics.
\textit{K. pneumoniae} have many virulence factors that make it pathogenicity, these factors are:

1. Capsule:
   \textit{K. pneumoniae} are typically enveloped by a polysaccharide capsule, which considered a major factor in the pathogenicity of these bacteria (5). Capsule protects the bacterium from phagocytosis by polymorphonuclear granulocytes and prevents killing of the bacteria by bactericidal serum factors on the other (6).

2. Adhesions or colonization factors:
   once bacteria enter the body of the host, they must adhere to cells of tissue surface, adherence which is only step in the infections process is followed by development of microcolonies and subsequent steps in the pathogenesis of infection(1). Pili (fimbriae) are non-flagellar, they are demonstrated mainly on the basis of their ability to agglutinate erythrocytes of different animal species, there are two predominant types in \textit{K. pneumoniae}:-
   - Type I pili or mannose-sensitive hemagglutination (MSHA).
   - Type 3 pili or mannose-resistant \textit{Klebsiella} hemagglutination (MR/K-HA) (7).

3. Siderophore production:
   \textit{K. pneumoniae} have evolved high-affinity iron assimilation systems called siderophores, which are small (MW 500-1000) ligands that are specific for ferric iron and thus supply iron to the bacterial cells (1). Moreover, Lewis (8) demonstrated that siderophore gene was colonized from the plasmids of some \textit{K. pneumoniae} strains and transferred to a non-virulent (siderophore negative) strains, on the other hand the production of siderophores resulting in enhanced growth in the host tissues.

The aim of this study are determine some virulence factors of \textit{K. pneumoniae} isolated from clinical sources.

Materials and Methods:

This study was carried out in General Al-Nasseryia Hospital, at Al-Nasseryia city. The specimens collected from patients undergoing surgical wound infections (their mean age was 45 years) and hospital environment with sterile cotton swabs were inoculated onto sheep blood agar (Mast diagnosis, England); MacConkey agar (HiMedia, India) plates and incubated aerobically for 24 hours at 37°C and they processed according to methods at standard procedure (9).

Identification of isolates was based on Gram stain; colony morphology, biochemical characteristics and confirm by API 20E system (Bio-Mérieux, France).

A total of 99 isolates of \textit{K. pneumoniae} were collected, which include: 38 isolates from hospital environment (surgical wards, operating theatres and hand of medical personnel) and 61 isolates were collected from wound infections of in-patients (admitted to hospital) undergoing surgical operation in the surgical wards. The control microorganism \textit{K. pneumoniae} (ATCC 13883) served as reference strains were obtained from Public Health Central Lab. Baghdad.

Detection of virulence factors of \textit{K. pneumoniae} as capsule: modified capsule stain (9); type I pili or MSHA (7); type 3 pili or MR/K-HA (7); and siderophores production (10).
Our data were analyzed statistically. Multiple Z test was used to test the significance of the relationships obtained. P value of more than 0.01 was considered significant.

Results:
The total isolates of *K. pneumoniae* which have some virulence factors were shown in Table 1. All strains were isolated from hospital environment have capsule (100%), MSHA (100%), MR/K-HA (100%) and siderophore production (100%).

Also, all isolates of *K. pneumoniae* were isolated from surgical wound infections have capsule (100%), MSHA (97%), MR/K-HA (100%) and siderophores production (98%).

Table 1: Some virulence factors of *K. pneumoniae* isolated from hospital environment and surgical wound infections.

<table>
<thead>
<tr>
<th>Virulence factors Isolates</th>
<th>The virulence factors</th>
<th>MZ-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capsule</td>
<td>MSHA</td>
</tr>
<tr>
<td>Hospital environment N=38</td>
<td></td>
<td></td>
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<tr>
<td>Surgical wound infections N=61</td>
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MSHA: Mannose –Sensitive Hemagglutination.
MR/K-HA: Mannose- Resistant *Klebsiella* Hemagglutination

MZ-test: Multiple Z test
OC: out of Comparision
NS: non-significant.

Discussion:
Some virulence factors were shown in Table 1.

*Capsule:
All isolates of *K. pneumoniae* have capsule. This is in agreement with Al-Saedi (11) and Muftin (12) who found 100% of *K. pneumoniae* have capsule. In the same sense, Podschan and Ulmann (4) demonstrated that *K. pneumoniae* usually have well- developed polysaccharide capsule, which give their colonies their characteristics mucoid appearance.

*Type I pili (MSHA):
From table 1, all isolates of *K. pneumoniae* which were isolated from hospital environment have type 1 pili, whereas 97% of isolates which were isolated from surgical wound infections have type 1 pili. There is no significant differences between them (p=1.128). This is in agreement with Al-Saedi (11) and Muftin (12) who found that all isolates of *K. pneumoniae* have type 1 pili.

*Type 3 pili (MR/K-HA):*

All isolates of *K. pneumoniae* have type 3 pili, our results showed out of comparison between isolates from hospital environment and surgical wound infections, this in agreement with Al-Saedi (11) and Muftin (12) who found that all the isolates of *K. pneumoniae* which were isolated from municipal hospitals have type 3 pili (MR/K-HA).

*Siderophores production:*

All isolates of *K. pneumoniae* were isolated from hospital environment produced siderophores, this is no significant differences between their (p=0.793), this is in agreement with the findings of Podschun et al. (13) who found that all isolates of *K. pneumoniae* which were obtained from clinical sources produced siderophores. Moreover, Podschun et al. (14) noted that when a bacterium can produce siderophores, if enables them to hemolyse erythrocytes on blood agar plates.

References:

دراسة بعض عوامل الضراوة للكلبسبيلا الرئوية المعزولة من مصادر طبية

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المستخلص:

أجرت هذه الدراسة في مستشفى الناصرية العام في مدينة الناصرية. إذ تمثل دراسة عدد من عوامل الضراوة لـ 99 عزلة من بيكتريا الكيسيللار النروية (61 عزلة من الجروح المخمجة و 38 عزلة من بينة المستشفى).

أظهرت الدراسة بأن جميع العزلات لهذه البكتريا تمثل المحفظة (100%)، 89% منها تمتلك النوع الأول من الاهلام أو عامل تجلط كريات الدم الحمراء الحساس كسكر المانوز، وتمتلك جميع العزلات (100%) النوع الثالث من الأهلام أو عامل تجلط كريات الدم الحمراء المقواوم لسكر المانوز، في حين أن 99% من هذه العزلات تستنتج نظام نقل الحديد (السيدرفورات).