DETECTION OF ADHESION GENES IN ENTEROBACTER SPP. ISOLATED FROM CLINICAL CASES IN AL-NAJAF CITY

Taif Al-Saadi¹, Sara Mohssin Yassir² Fadhil Faez sead, Mona Adel Ismail¹ and Faiz Kamil katab³

ABSTRACT:

Background and Aim of the study: Enterobacter spp. is also an important nosocomial pathogen that causes bacteremia and infections of the lower respiratory tract, urinary tract, and burns, as well as endocarditis, septic arthritis, and skin and soft tissue infections. A sample (204) was isolated from different cases and these isolates distributed by 98 isolated from urine, 42 isolated from wound, 35 isolated from diabetic foot, 11 isolated from vaginal swab and 18 isolated from burns in Al-Najaf city (Al-Sadr Medical City, AL-Forat Hospital) and identification of bacterial isolates was performed using biochemical testing and Vitek 2 system, PCR amplification technique was used to investigation of predominance, showed that (23) isolates were Enterobacter spp. After incubation periods, the results showed that (77.07%) samples showed bacterial growth and (22.92%) showed no growth. Enterobacter spp. identified in (29.84%) the bacterial growth adhesion genes and fimH and the aggregation genes esgA and csgD among Enterobacter spp.

Results: The results of PCR breeding technique showed that (26%) and (17%) bacterial isolates carried fimH and fimA genes, while (13%) bacterial isolates carried esgA and csgD genes.

Keywords Enterobacter spp., FimA, FimH, Nosocomial infection.

INTRODUCTION:

Nosocomial infection NI, which is called Infections acquired in hospitals is described as localized or systemic infection transport from the hospital after 48 hours of residency or from the patient, admitted for any other reason the specific infection. Nosocomial infections are caused by varieties of microorganisms (bacteria, fungi, viruses, and parasites) but approximately 90% of Nosocomial infection are due to bacteria. Nosocomial infection is an infection that is obtained after admission of patients but not presents through the entry (¹). Nosocomial infections arising from anybody site can lead to sepsis, organ failure, and death. The stomach is typically a sterile environment because of the combined activities of salivary flow, mucus, secretory IgA, gastric acid, bile, and peristalsis. Most nosocomial urinary tract infections occur secondary to the use of urinary catheters. Infections of the bloodstream can occur secondary to bacterial trans-location across gut mucosa, wound infections, or infections at anybody site. Infection of surgical wounds requires introduction of bacteria, but the number of bacteria necessary to cause infection depends on the condition of the wound. General strategies for the prevention of nosocomial infection involve strict adherence to hygiene (²). Gram-negative rods that are facultatively anaerobic and often encapsulated, Enterobacter spp. are primarily the main cause of urinary and respiratory tract infections (³). Enterobacter spp. is one of the most common gram-negative pathogens associated with nosocomial infections, accounting for 6% of nosocomial isolates.
recovered and 11% of pneumonia isolates (4). Although 25% were resistant to broad-spectrum cephalosporins, Enterobacter spp. still very unusual. Enterobacter cloacae complex (ECC) has become an important pathogen commonly encountered in nosocomial infections.

MATERIAL AND METHODS:

During the study period from May to August 2023, (204) samples were collected from clinical samples including urine (98), vaginal swabs (11), wounds (42), burns (18) and diabetes feet (35). All samples were transported to the laboratory and cultured on MacConky agar for 24 h at 37°C. After the incubation period, identification of bacterial isolates depends on culture characteristics and biochemical tests, final identification is performed using the Vitek-2 system. PCR amplification was used to determine the presence of the FimA, FimH, csgA and csgD genes. The primer used in this study was.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FimH</td>
<td>F: ATGAAACCGCTTGGCTCTTGCG R: GCTGAACGCCTATCCCCTGC</td>
<td>Fertas et al., (2013)</td>
</tr>
</tbody>
</table>

Each 25 µl PCR reaction mixture for PCR contains 2.5 µl upstream primer, 2.5 µl downstream primer, 2.5 µl free nuclease water, 5 µl DNA and 12.5 µl finished PCR tube master mix. thin. The conditions of the thermal cycler were as follows:

<table>
<thead>
<tr>
<th>PCR gene</th>
<th>Initial denaturation</th>
<th>Cycling condition</th>
<th>Final extension</th>
<th>Cycle number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fim H</td>
<td>95 °C/4min</td>
<td>Denaturation: 95 °C/30sec</td>
<td>Annealing: 53 °C/1min</td>
<td>Extension: 72 °C/1min</td>
</tr>
<tr>
<td>Fim A</td>
<td>94 °C/4min</td>
<td>Denaturation: 94 °C/1min</td>
<td>Annealing: 59 °C/1min</td>
<td>Extension: 72 °C/30sec</td>
</tr>
<tr>
<td>csgA</td>
<td>94 °C/4min</td>
<td>Denaturation: 94 °C/1min</td>
<td>Annealing: 56 °C/1min</td>
<td>Extension: 72 °C/30sec</td>
</tr>
<tr>
<td>csgD</td>
<td>94 °C/4min</td>
<td>Denaturation: 94 °C/1min</td>
<td>Annealing: 54 °C/1min</td>
<td>Extension: 72 °C/30sec</td>
</tr>
</tbody>
</table>

Ethical consideration:

Ethical approval was granted by Al-Najaf Hospitals Consent form was obtained from the patients for the purpose of this control case study. 887/27/7 [14/3/2023].

RESULTS:

Enterobacter spp. isolated from various clinical samples using specific media such as MacConkey agar and CHROM™. Isolates were also diagnosed using the Vitek2 system using GN/ID identification cards for diagnosis and the results confirmed only 23 isolates from clinical cases are Enterobacter spp. as following E. cloacae spp. cloacae 9 isolates, E. cloacae complex 6 isolates, E. aerogens 8 isolates from clinical case, the table below shows the distribution.
Detection of Adhesion Genes in Enterobacter Spp.

Table (1): Isolation of Enterobacter spp. from clinical cases.

<table>
<thead>
<tr>
<th>source</th>
<th>Type of Enterobacter species</th>
<th>E. cloacae spp cloacae</th>
<th>E. cloacae complex</th>
<th>E. aerogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td></td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Wound</td>
<td></td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>diabetic foot</td>
<td></td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>vaginal swab</td>
<td></td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Burns</td>
<td></td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

A total of 23 Enterobacter spp. isolates collected from clinical cases Table (1), were molecularly tested for the presence and distribution of FimH found in 6 (26%) Figure(1), while FimA showed 4 (17%) Figure (2). The results of fimH disappearing from Enterobacter spp. in the present study. Research results showed that csgA and csgD were found in 3 (13%) isolates. Curli fimbria, encoded by csgA, is an important factor for cell adhesion, aggregation, and biofilm formation in many Enterobacteriaceae.

Figure 1: Gel electrophoresis of PCR amplified product of fimH gene primers with product 508 bp of Enterobacter spp. isolates

Figure 2: Gel electrophoresis of PCR amplified product of fimA gene primers with product 434 bp of Enterobacter spp. isolates
Figure 3: Gel electrophoresis of PCR amplified product of csgD gene primers with product 385bp of Enterobacter spp. isolates

The fimH gene results of Enterobacter spp. in present study are agreement with (6) obtained that 32 Enterobacter spp. isolated from clinical urine specimens and identified the fimH gene in 40% of the isolates. Additionally, (7) isolated 8 E. cloacae identified the fimH gene, while the(8) show high result 18(75%) of Enterobacter spp.

Figure 4: Gel electrophoresis of PCR amplified product of csgA gene primers with product 276 of Enterobacter spp. isolates

DISCUSSION:

The fimbriae play an important role in connecting epithelial cells. Fimbriae participate in bacterial colonization and host cell restriction signaling to explicit host receptors, the results of fimH disappearing from Enterobacter spp. in the present study, the results with (6) agree that 23 Enterobacter spp. isolated from clinical urine samples and identified the fimH gene in 40% of isolates. Furthermore, (7) isolated 8 E. cloacae identified the fimH gene, while (8) gave a high result of 18(75%) Enterobacter spp. who collected 24 strains of Enterobacter spp. in the city of Babylon.

Fimbriae attachments control both the fate of the bacterial macrobiotic within the
Detection of Adhesion Genes in Enterobacter Spp.

host and the movement of the disease cycle. The destructive nature of organic entities is not entirely regulated by type 1 fimbriae of fimA (9&10). The presence of fimA, which encodes a type 1 fimbria glue structure, allows bacteria to invade and create biofilms while also blocking the entry of antibiotics into cells. The connection of Enterobacteriaceae to epithelial and endothelial cells is supported by type one and type three fimbria (11&12).

One of the most important harmfulness variables of E. cloacae is Curli fimbriae because of its activity in biofilm arrangement. The pathogenesis of this microorganisms relies basically upon their ability to communicate and making of curli fimbriae which is remembered for cells assortment connection to foundation, and creation of biofilm, likewise is considered as important agent that cooperate with have proteins, recommended to assist spreading of microorganism, in the host (13&14). The protein csgD, for "Curlin subunit quality D," is a transcriptional controller that manages various qualities engaged with the Curli gathering, transport, and primary parts, which are significant for biofilm development (15).

Conflicts of interest:

No conflicts of interest

REFERENCE:

1. Fürnkranz, Ursula, and Julia Walochnik. "Nosocomial infections: Do not forget the parasites!" Pathogens 10.2 (2021)


**Enterobacter spp.**

Muaz’il min al-halalat al-sirrīyah

مُعْزِزَةً مِنِ الحَالَاتِ السَّرِيرِيَّة

الطيف رزاق مجید١، سارة محسن ياسر٢، فاضل فائز سعيد٣، فانز كمال كتاب٣

قسم تقنيات المختبرات الطبية - المعهد التقني - جامعة الفرات الأوسط التقنية – كوفة – العراق١

الجامعة الإسلامية – النجف٢

مستشفى الفرات الأوسط٣

"الكشف عن جينات الالتصاق في **Enterobacter spp.** في مدينة النجف الاشرف."

"才发现粘附基因在**Enterobacter spp.**在巴格达市。

"طيف رزاق مجيد١، سارة محسن ياسر٢، فاضل فائز سعيد٣، فانز كمال كتاب٣

قسم تقنيات المختبرات الطبية - المعهد التقني - جامعة الفرات الأوسط التقنية – كوفة – العراق١

الجامعة الإسلامية – النجف٢

مستشفى الفرات الأوسط٣

الأمنية الطبية. وهو أيضاً أحد مسببات الأمراض الهامة في المستشفيات الموكلة عَرَضة الدم والجهاز التنفسي، وامثال البول والازدحام، بالإضافة إلى التهاب الشغاف الداخلي والتهاب المفاصل الإضافية والتهابات الجلد والأنف والأنف والأنف ونظام Vitek-2 ونظام Vitek-2.

عزلت عينة من حالات مختلفة في مدينة النجف ونصحة الغارتين البكتيرية باستخدام الاختبار الكيميائي ونظام Vitek-2 ونظام Vitek-2.

بعد فحص العينة، أظهرت نتائج PCR على أن (77.07%) من العينات أعطت نموًا، بينما (22.92%) لم تظهر أي نمو. انتشرت الأحماض النيابة المضادة (71.98%) من المولد البكتيري وجينات الالتصاق fimA و fimH و csgA و csgD Enterobacter spp. • csgA

أظهرت نتائج تقنية PCR (26%) وأظهرت نتائج تقنية PCR (17%) من العينات حملت جينات البكتيرية، بينما (13%) من العينات.