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

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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.

Primer	Sequence	Reference
FimA	F: GCACCGCGATTGACAGC R: CGAAGGTTGCGCCATAG	Ghasemian *et al*., (2019)
FimH	F: ATGAACGCCTGGTCCTTTGC R: GCTGAACGCCTATCCCCTGC	Fertas *et al*., (2013)
csgA	F-TTCAAAGTGGCAGITATTGCAG R-TTTTTGCAGCAGATCGATAGAA	Kim SM et al (2012)
csgD	F-GAAATTGCATAATATTCAACGTTC R-TITGTTCAGGATCTCTTTTTCAC	Kim SM et al (2012)

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:

PCR gene	Temperature (c) / Time					Cycle number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
Fim H	95 ˚C/4min	95 ˚C/30sec	53 ˚C/1min	72 ˚C/1min	72 ˚C/8min	35
Fim A	94 ˚C/4min	94 ˚C/1min	59 ˚C/1min	72 ˚C/30sec	72 ˚C10min	30
csgA	94 ˚C/4min	94 ˚C/1min	56 ˚C/1min	72 ˚C/30sec	72 ˚C10min	30
csgD	94 ˚C/4min	94 ˚C/1min	54 ˚C/1min	72 ˚C/30sec	72 ˚C10min	30

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.

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

source	Type of *Enterobacter* species		
	E.cloacae spp cloacae	*E. cloacae complex*	*E. aerogenes*
Urine	4	3	3
Wound	1	2	2
diabetic foot	2	-	2
vganial swab	1	1	_
Burns	1	-	1
Total	9	6	8

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*.

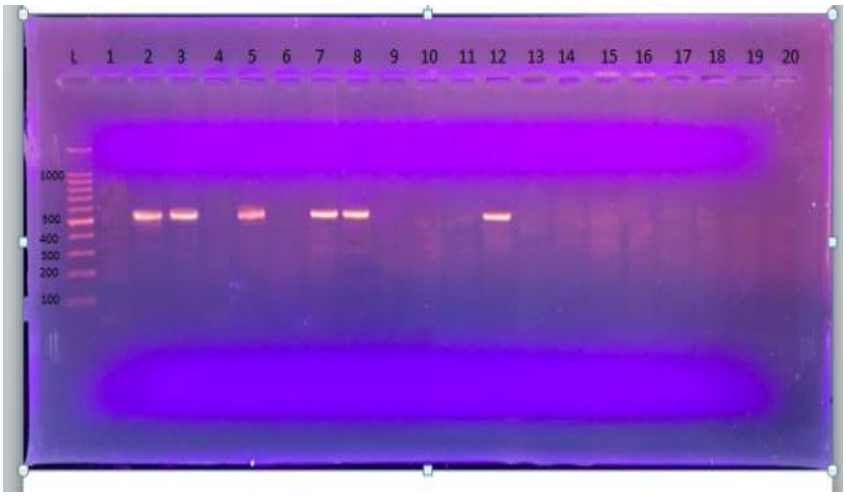

Figure 1: Gel electrophoresis of PCR amplified product of *fimH* gene primers with product 508 bp of *Enterobacter spp*. isolates

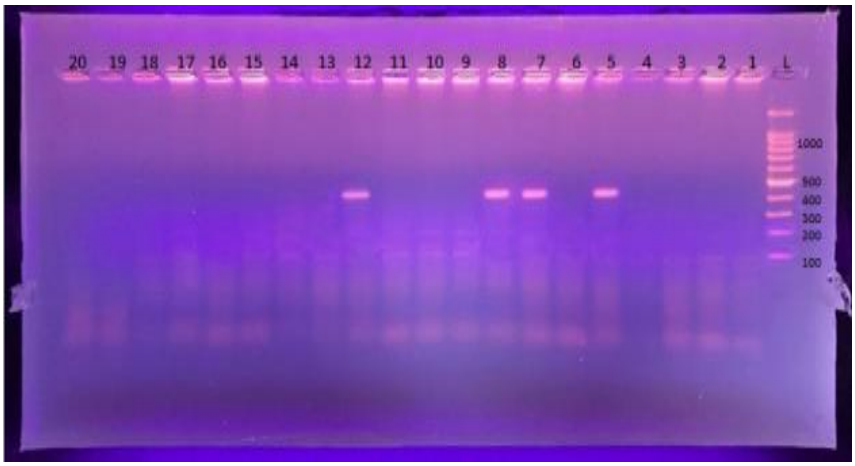

Figure 2: Gel electrophoresis of PCR amplified product of *fimA* gene primers with product 434 bp of *Enterobacter spp*. isolates

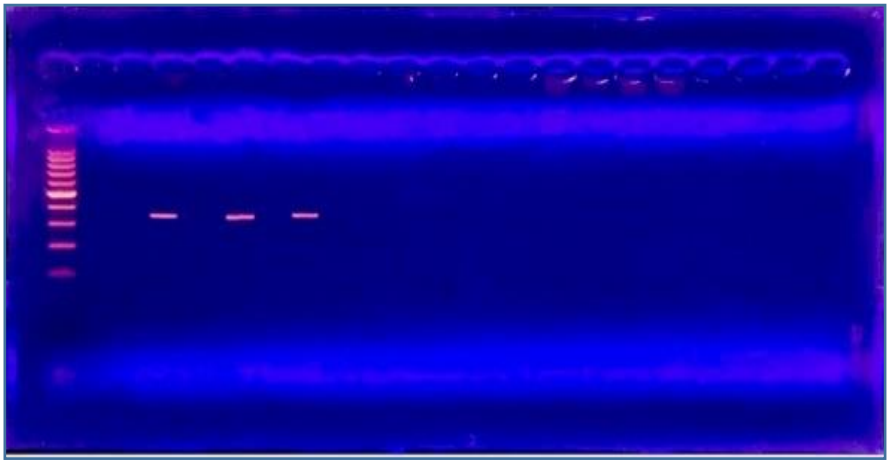

Figure 3: Gel electrophoresis of PCR amplified product of csgD gene primers with product 385bp of *Enterobacter spp*. isolates

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.* who collected 24 isolate from *Enterobacter spp*. in Babylon city .The results of study revealed that *csgA* and *csgD* Figure (3) Figure (4)were found in 3 (13%) of isolates. Curli fimbria, encoded by *csgA*, is an important factor for cell adhesion, aggregation, and biofilm formation in many *Enterobacteria* [9].

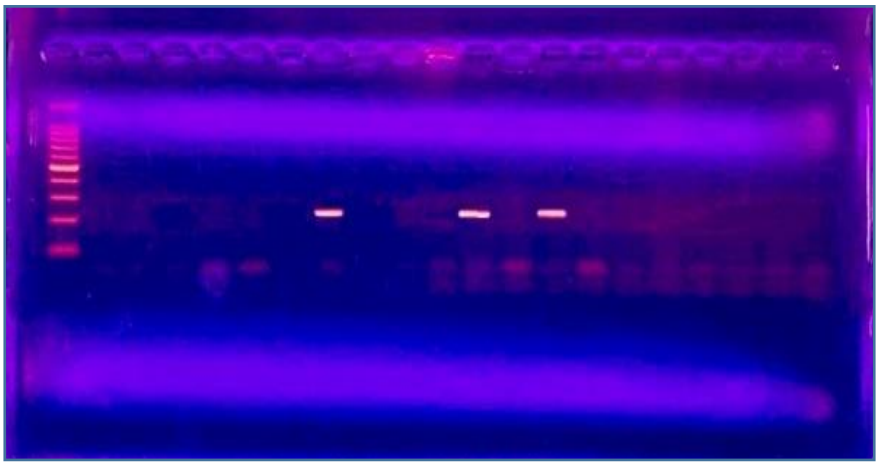

Figure 4: Gel electrophoresis of PCR amplified product of csgA gene primers with product 276 of *Enterobacter spp*. isolates

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

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

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الكشف عن جينات الالتصاق في *Enterobacter* spp. معزول من الحالات السريرية في مدينة النجف الاشرف

طيف رزاق مجيد[1] ، سارة محسن ياسر[2]، فاضل فائز سعيد[2]، منى عادل اسماعيل[1]، فائـز كامل كتاب[3]

قسم تقنيات المختبرات الطبية - المعهد التقني - جامعة الفرات الاوسط التقنية – كوفة – العراق 1
الجامعة الاسلامية – النجف 2
مستشفى الفرات الاوسط 3

الأمعائية النيابة. وهو أيضًا أحد مسببات الأمراض الهامة في المستشفيات المسؤولة عن تجرثم الدم والجهاز التنفسي السفلي والمسالك البولية والحروق، بالإضافة إلى التهاب الشغاف الداخلي والتهاب المفاصل الإنتاني والتهابات الجلد والأنسجة الرخوة.

عزلت عينة من حالات مختلفة في مدينة النجف وشخصت العزلات البكتيرية باستخدام الاختبار الكيموحيوي ونظام2-Vitek ، واستخدمت تقنية تضخيم PCR لتقصي السيادة، وتبين أن (23) عزلة هي Enterobacter spp. بعد فترة الحضانة أظهرت النتائج أن (77.07%) من العينات أعطت نمواً بكتيرياً و (22.92%) لم تظهر أي نمو.

الأمعائية النيابة. تم التعرف على (29.84%) من النمو البكتيري وجينات الالتصاق fimA و fimHوجينات التجميع csgA وcsgD Enterobacter spp.

أظهرت نتائج تقنية تكاثر PCR أن (26%) و(17%) من العزلات حملت جينات fimH وfimA، بينما (13%) من العزلات البكتيرية كانت تحمل جينات csgA و.csgD