Molecular Detection of Aggregation and Adhesion Genes of Enterobacter cloacae isolated from Gall-Bladder Infections

Kahlaa Muhammed Abbas Al-Mulla [1,2]And, Abbas Shaker Al-Muhanna [2]
[1]College of Health and Medical Techniques/Al-Furat Al-Awsat Techniques University, Kufa, Iraq
[2]College of Science, University of Kufa, Iraq.
*Corresponding Author, Email: kh2030khk@gmail.com

Abstract
Background: *Enterobacter cloacae* is also an important nosocomial pathogen responsible for bacteremia and lower respiratory tract, urinary tract and intra-abdominal infections, as well as endocarditis, septic arthritis, osteomyelitis and skin and soft tissue infections. Introduction: Acute cholecystitis (AC) is one of the most common surgical diseases. Bacterial infection accounts for 50% to 85% of the disease's onset. Since there is a close relationship between the biliary system and the gut, the aims of this study were to characterize and determine the influence of gut microbiota on AC, Methodology: 174 clinical specimens (swab) were collected from patient suffering from gall-bladder infection, after the specimens were cultured on suitable media, *the* identification of bacterial isolates was carried out using biochemical test and Vitek-2 system, PCR amplification technique were used to investigation the predominance, aggregation genes *csg*A and *csg*D and adhesion genes *fim*A and *fim*H among *E cloacae* isolate Results: After incubation period the results appear that (47.12%) of specimens were gave bacterial growth and (52.88 %) were appear no growth. *E cloacae* were identified in (24.39%) of bacterial growth., the results PCR multiplication technique appear that (40%) and (10 %) of isolates carried *csg*A and *csg*D genes, while (15%) and (35%) of bacterial isolates were carrying *fim*A and *fim*H genes. Conclusions: Bacteria the major agent of gall-bladder infections and E cloacae was the most common bacteria causing gall-bladder infections.
Keywords *Enterobacter cloacae*, csgA , *csgD,* Gallbladder, virulence factors

1. Introduction

Gallbladder is a pear-shape organ to digestive storage; located below the liver and upper right side of the abdomen;(1). Its working for store and gradually releases bile for fats digestion, (2). Cholelithiasis is the presence of gallstones in gallbladder or in the bile duct, it's the inflammation of gallbladder that caused by obstruction of the biliary tract and may be acute, chronic, or acute superimposed on chronic; and almost occurs in association with presence the gallstones (3). Different microbiological and molecular methods have been showed the presence of different bacteria in gallbladder or in hepato-biliary tree such as *Escherichia* spp., *Enterococcus spp. Streptococci* spp., *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Salmonella* spp. and *Acinetobacter* spp. (4). Bacteria have been implicated in the pathogenesis of gallstones, producing high levels of beta glucuronidase, biliary mud, and phospholipases A2 (5). Due to the limitation of culture conditions, most bile bacterial isolates from clinical samples are aerobic bacteria especially Gram stain-negative Enterobacteriaceae, such as *Escherichia coli, Klebsiella spp., Enterobacter* spp., and less frequently anaerobic bacteria, including *Bacteroides* and *Clostridium* spp., as well as microaerophilic *Helicobacter pylori* (6,7)
Enterobacter is a genus belong to the family of *Enterobacteriaceae* that is associated primarily with healthcare-related infections. There are currently 22 species. However, not all species share known to cause human disease, *Enterobacter* species are responsible for causing many nosocomial infections, less commonly community-acquired infections, including urinary tract infections (UTI), respiratory infections, soft tissue infections, osteomyelitis, and endocarditis. *Enterobacter* species can be present in human skin surfaces, water, certain foods, soil, and sewage. (8).
The *Enterobacter cloacae* is a common nosocomial bacterium that can cause a variety of illnesses among human involve pneumonia, urinary tract infections and septicemia (9).
The study aim to detected the predominance of aggregation and adhesion gene of Enterobacter cloacae in bacterial gall-bladder infection.

2. Materials and Methods

During study period from July to November 2022, 174 swab were collected from patients suffering from gallbladder infection (after cholecystectomy) from both female and males in different ages. The specimens were cultured on suitable media (MacConkey, Blood and Mannitol agar), after incubation period the identification of bacterial isolates depend on cultural characters and biochemical test, and the final identification were carried out using Vitek-2 system. Primers used in this study and PCR program applied in the

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thermo-cycler are shown in Tables 1 and 2, respectively.

Table 1: Primers used in this study

Gene	Primer sequence (5′ to 3′)	Product size (bp)	Reference
csgA	F- TTCAAAGTGGCAGTTATTGCAG R- TTTTTGCAGCAGATCGATAGAA	276	Kim SM et al(2012)
csgD	F- GAAATTGCATAATATTCAACGTTC R- TTTGTTCAGGATCTCTTTTTCAC	385	Kim SM et al(2012)
FimA	F:GCACCGCGATTGACAGC R:CGAAGGTTGCGCCATAG	132	Ghasemian *etal.*,(2019)
FimH	F:ATGAACGCCTGGTCCTTTGC R:GCTGAACGCCTATCCCCTGC	508	Fertas et al.,)2013(

Table 2: PCR Program Applied in the Thermo-Cycler.

Final extension	Extension	Annealing	Denaturation	No. of cycles	Initial Denaturation	Gene
72°C for 10 min	72°C for 30 sec	56	94°C	30 cycle	94°C for 4Min	*csgA*
72°C for 10 min	72°C for 30 sec	54	94°C	30 cycle	94°C for 4Min	*csgD*
72°C for 10 min	72°C for 30 sec	59	94°C	30 cycle	94°C for 4Min	*FimA*
72°C for 10 min	72°C for 30 sec	53	94°C	30 cycle	94°C for 4Min	*FimH*

3. Results and Discussion

Cholecystitis leads to gallbladder inflammation or even perforation, tissue death, gangrene, fibrosis, and shrinking of the gallbladder, which is a hospitalized disease with increasing medical and financial burden bacteria, as one of the important risk factors, were closely correlated with its poor operative outcomes. (10,11). For this resented, the study amid to investigated the role of bacteria in gallbladder infections, particularly *Enterobacter cloacae.*

After incubation period the results indicated that 82 (47.12%) gave bacterial growth, and 92(52.88%) appear no growth ,63 (76.82 %) of bacterial isolates was recovered from female and 19 (23.18%) from male.

The primary identification of bacterial isolates according cultural and biochemical test ,while finally identification carried out using Vitek_2 system ,the results appear that 16 (19.51%) were identified as *Staphylococcus* spp. represented by ,2 of isolates were *S werrine* , 3 of isolates were recorded for each of *S hominis* and *S lugdunsis* ,while 4 of isolates were identified for each of *S haemolyticus* and S *epidermidis* , 66 (80.48 %) were identified gram negative bacteria ,which were represented by 4 of isolates were *Klebsiella pneumonia* , 3 isolates were *K.oxytoca* , *Psudomonas aeruginosa* and *P fluorescence* were recorded in 4 and 5 of isolates respectively. *Salmonella* were recorded in 7, *Serratia marcescIns* were found in 4 isolates, while ,*Achromobacter xylosoxidnt* , *Acinetobacter baumanii* , and *Sphengomonas pancimobilus* were recorded in 2 of isolates for each one , *Enterobacter cloacae* were identified in 20 isolates and 8 of isolates were found *E coli* .

Molecular detection of virulence factors of *Enterobacter cloacae* was studied using PCR technique with specific primer. the results of study revealed that *csg*A were found in 8 (40%) of isolates. Curli fimbria, encoded by *csg*A, is an important factor for cell adhesion, aggregation, and biofilm formation in many enterobacteria (12).

(11) found in their studs that 59% of isolates were carried csgA gene, while (13) found in their study that all isolates (77.7%) harbored csgA gene in their genome. The results of PCR amplification for *csg*D gene found that the gene were recorded in 2 (10 %)of bacterial isolates ,The protein *csgD*, for "Curlin subunit gene D," is a transcriptional regulator that regulates a number of genes involved in the Curli assembly, transport, and structural components, which are important for biofilm formation .Previous study of (14) fount that *csg*D gene were detected in (66.6%) of *Enterobacter cloacae* isolates .(11) recorded in their study that (68.4%)of bacterial isolates were carried *csg*D gene .while(15) reported in their study that (5%) of bacterial isolates were carried *csg*D gene .

The result of study revealed that 3 (15%) of isolates have *fim*A gene, which encoded for adhesion factors type 3 fimbria. (16) fond in their study that *fim* A gene were found in (45%) of isolates, While (17) found that (30%) of isolates were carried *fim* A gene.

In present study *fim*H gene were detected in 7(35%) of isolates, which encoded *FimH*-mediated auto aggregation and biofilm formation may enable pathogen to better withstand antibiotic treatments and host antibacterial defense. In addition, type 1 pilus mediated biofilm formation may facilitate bacterial colonization of urinary catheters and other medical implants, an unfortunately common problem for hospitalized individuals. (18) pointed out in their study that *fim*H gene were detected in (75%) of E cloacae isolates .while, (17) found that (50%) of isolates were carried *fimH* gene in their genome ,which enable bacteria to aggregation and formation biofilm (Figures 1-4).

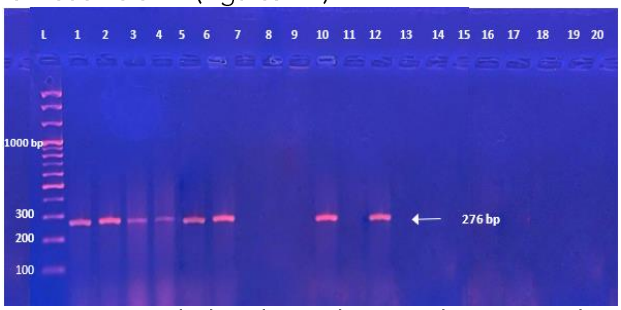

Figure 1: Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of Enterobacter cloacae using primer csgA gene with product 276 bp. The electrophoresis was performed at 70 volt for 1.5. lane (L), DNA molecular size marker (100- 1000 bp ladder). Lanes (1,2,3,4,5,6,10,12) show positive results with csgA gene.

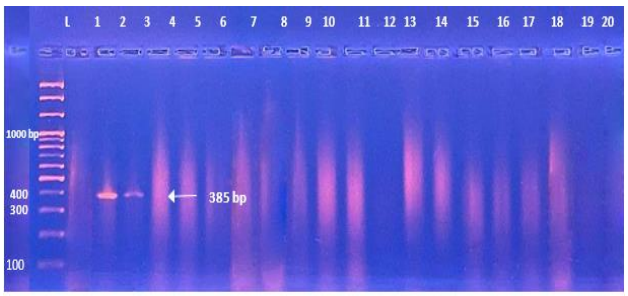

Figure 2: Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of Enterobacter cloacae using primer csgD gene with product 385 bp. The electrophoresis was performed at 70 volt for 1.5. lane (L), DNA molecular size marker (100- 1000 bp ladder). Lanes (2,3) show positive results with csgD gene.

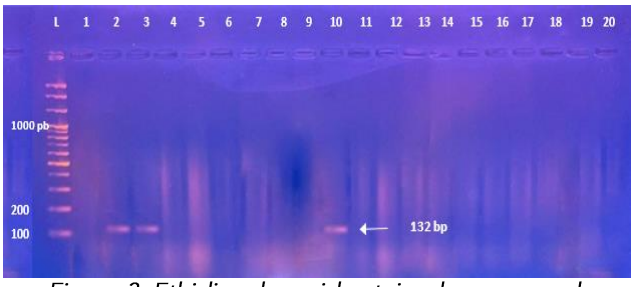

Figure 3: Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of Enterobacter cloacae using primer fimA gene with product132 bp.The electrophoresis was performed at 70 volt for 1.5. lane (L), DNA molecular size marker (100 -1000bp ladder). Lanes (2,3,10) show positive results with fimA gene.

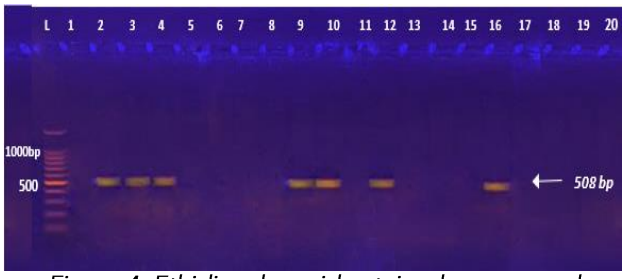

Figure 4: Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of Enterobacter cloacae using primer fimH gene with product 508bp.The electrophoresis was performed at 70 volt for 1.5. lane (L), DNA molecular size marker (100 -1000bp ladder). Lanes (2,3,4,9,10,12,16) show positive results with fimH gene.

4. Conclusion

-Bacteria have big role in causing gall-bladder infection. Enterobacter cloacae is important bacterial agent of gall-blader infections. PCR amplification was detected aggregation genes *csg*A and *csg*D in (40%) and (10%) of E cloacae isolates ,while adhesion genes *fim*H and *fim*A in (35%) and (15%) of isolates.

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