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Molecular detection of Curli biogenesis genes in Enterobactercloaecae complex isolated from irritable bowel syndrome patients

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Abstract
Background: Enterobacter cloacae complex (ECC) represent a pathogen belonging to "small intestinal bacterial overgrowth" (SIBO) complicated in manifestation of irritable bowel syndrome. Curli fimbriae are formed on the extracellular layer of numerous ECC where they enable pathogen on attachment and biofilm progress. Aim: to prove the relationship between curli fiber in *E. cloacae* as main virulence factor and IBS occurrence. **Patients and methods:** Fluid aspirates of third part of the duodenum were collected from fifty IBS patients, these specimens were seeded on MacConkey agar and blood agar media to isolate ECC species, which identified by Vitek 2 system. The existence of csgAB operon, csgA and csgD genes was detected by conventional PCR of csgAB, csgA and csgD definite primers. **Results:** seven of *E. cloacae* isolates were recovered from fifty duodenal aspirates at a percentage 14%. All the *E. cloacae* isolates (100%) revealed positive results for csgAB operon, csgA and csgB genes. **Conclusion:** Duodenal aspirates *E. colacae* possession of curly biogenesis genes helps them form the biofilm This contributes to patients not responding to antibiotics.

Key words: Irritable bowel syndrome, Enterobacter cloacae complex, curli fimbriae

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Introduction

"Irritable bowel syndrome" (IBS) is a common dysfunction of gastrointestinal tract defined by Pain and flatulence, constipation, and/or diarrhea, that may be activated by enteric pathogens and has also been linked to alterations in the microbiota [1] nutrition sensitivity, carbohydrate lack absorption, and inflammation of the mucosal layer of intestine all have been involved in the pathogenesis of IBS [2].

Small intestinal bacteria1 overgrowth (SIBO) is one appearance of gut microbiome disturbance and is highly prevalent in IBS [3]numerous researches issued the past few years proved an correlation between(SIBO) and (IBS) [4-5], in SIBO The normal intestinal flora of the duodenum (proximal intestine at the stomach) is changed both in quantity and quality, where the unfriendly bacterial presence in the small intestine above 10^5 -10^6organisms/ml. where it typically are less than 10^3organisms/ml[6], and the bacterial community is low of Gram-negative bacteria,the common of Gram-positive bacteria while anaerobes are rare [7].

The symptoms of IBS, as a consequence of SIBO fermentation activity of dietary carbohydrates which leads to increase production of gas [5]. Pistiki et al 2013 [8] isolated E. cloacae from duodenal aspirates as a member of SIBO Enterobacter cloacae is gram negative opportunistic pathogen. It is One of the most common Enterobacter species involved in numerous systemic and localized infections, such as bacteremia ,urinary tract infections (UTI), gastrointestinal tract (GIT) infections , injury infections and contamination of hospital instruments like catheters[9 -10]. Curli fiber is a novel class of bacterial external structures which is definite in E. cloacae , Escherichia coli , and Salmonellae spp. it represent a main virulence factors associated with adhesion to surface ,cell accumulation and biofilm development[11] .

The genes of curli biogenesis are assembled in two operons the csgBA and csgDEFG they encodes seven proteins CsgA, CsgB, CsgC, CsgD, CsgE, CsgF, and CsgG.CsgA is the major curli fiber subunit, . CsgB is a nucleator factor for nucleating CsgA subunits into fibers and supports the fibers to fix on the cell surface while the other proteins csgE-G represent curli export machinery forms a complex channels found in the outermembrane which recognize curli subunits and effort them(Csg-A

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and Csg-B)from periplasm into extracellular milieu [12]. The expression of csgBA (C) operon is orderly formed by the regulatory curli protein Csg-D from Csg-DEFG operon. [13]

Material and methods

Patients and Specimens collection

To investigate curli fimbriae genes in *Enterobacter clocaea* isolates we need primary isolates of fluid aspirates from third part of the duodenum because subculture of isolates lack curli fimbriae virulence factor [5]. So fifty fluid aspirates of third part of the duodenum were collected during the period October 2019 to January 2020 from irritable bowel syndrome (IBS) patients age range (20-50 years) which undergoing .Upper Gastrointestinal tract endoscopy in the Gastrointestinal tract unit in Marjan medical city, Iraq. Every patient was allowed to be enrolled as participant in the study once after written informed agreement about, name, medical history, intake treatment, age, types of specimen, the exclusion criteria include patients with gastric ulcer, IBD, UC, celiac disease.Duodenal aspirates were immediately transported to the bacteriological laboratory were inoculated on MacConkey agar plates and blood agar and aerobically incubated overnight at 37°C.

Enterobacter cloacae (*E. cloacae*) complex species identification

All the seven *E. cloacae* complex species were identified with Vitek 2 system for Gram negative bacteria (Biomerieux-France).

Molecular detection of curlifimbriae biogenesis genes

The bacterial Chromosomal DNA was extracted for PCR amplification had been used the guideline supplied by the scientific institute of the Wizard genomic DNA extraction kit (Promga, USA).The existence of "csgBA operon, csgA and csgD" were determined by using restricted primers for csgBA(C),csgA and csgD genes respectively.The details of primers showed in Table-1

Table -1: primer details used in the study

Target gene	Primer sequence(5-3)	Replicon size(bp)	reference
csg AB	F: ATGATGTTAACAATACTGGGTGC R: CGGCCATTGTTGTGATAAAG	1300	Kim et al.(2012)
csgA	F: ATTGCAGCAATCGTAGTTTCTGG R: ATWGAYCTGTCATCAGAGCCCTGG	245	Akbari et al.(2015)
csgD	F: TGAAARYTGGCCGCATATCAATG R: ACGCCTGAGGTTATCGTTTGCC	355	Akbari et al.(2015)

The total volume of PCR was 25 µl, which contained forward primer 1µl and the reverse primer 1µl, 5µ1 of template DNA specimen, 12.5µl of master mix (Promega-USA) and 5.5µ1 of Nuclease free-water. The PCR amplification was achieved with the thermo cycler PCR machine for csgBA(C) as following: for denaturation at 95ºC for 5 min, l cycle followed by 35 cycles of 94°C for l min, 57°C for l min, and 72°C for l min; a final extension step for l0 min at 72°C; while the cycling conditions for both csgA and csgB as a following: denaturation for 5 minutes at 95°C; 35 cycles of 95°C for l5 second, 60°C for l5 second, and 72°C for l5 second; extension for 5 minutes at 72°C. Successful PCR replicon size was established by 1.5% agarose gel electrophoresis at 80 Volts for 50 min staining with ethidium bromide under UV light, with DNA marker 100-1500bp.[14]

Results

The results of the current study explained that out of 50 duodenum fluid aspirate samples, only 5 samples are positive for the E.cloacae complex spp. with a percentage of 14%. All the seven *E. cloacea* complex isolates diagnosed by Vitek 2 system had bionumber (0627634553532010) at 99% identity with *E. cloacea* complex species. According to the PCR amplification profiles, all the *E. cloacea* strains (100%) showed positive results for csg AB(C) operon, csgA and csgB at a replicon sizes 1300 bp; 245 bp and 355 bp respectively. Figures -1: A, B, C.

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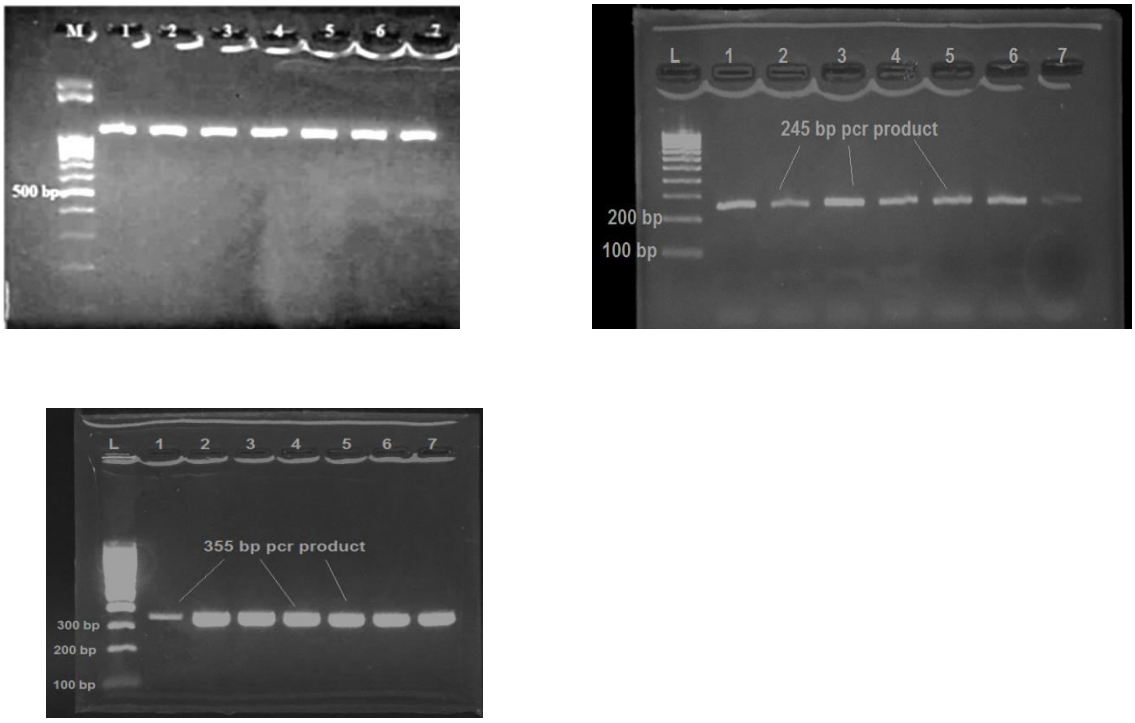

Figure1: agarose gel electrophoresis of PCR products visualized under U.V light after staining with thidium bromide, M: refer to DNA size marker, 100-2000 bp lane, 1-7 refer to the number of samples; (A) of csgAB operon at expected band 1300bp ; (B) of csgA gene at expected band 245bp and (C) of csgD genes at expected band 355bp

Discussion

One of the most common hypotheses about the etiology IBS is that mild inflammation and /or altered intestinal fecal microbiota in intestine [15]. *E. colacae* is a member of small intestine bacterial overgrowth (SIBO) associated with IBS as demonstrated by [5] who, Found that duodenal aspirate culture by 13.2% of *E .cloacae* in IBS patients

As well The scope of the present study includes isolation of *E. cloacae* from IBS 'patients which was 14% similar with those conducted by other study[5] , with study of main virulence factors associated with formation of biofilm (Curli fibers) Similar disease was studied by Linninge et al,2018 [16] about diverticular disease (DD),he disease has similar symptoms and it is public GIT disease of unidentified etiology , the same authors that Patients with Diverticular disease (DD) had increase number of bacterial species belong *Enterobacteriaceae* in the mucosal microbiota compared to patients with health subject. Other study submitted by [17], who found that , the most frequency isolates in SIBO cases of IBS were *Escherichia coli*, Enterococcus spp and *Klebsiella pneumonia*. All the *E. cloacae* isolates in the current study contain csgAB operon, csgA, and csgD genes as shown by the PCR amplification results

In Iran study [14] found that all bloodstream *E. cloacae* (100%) harbored csg D gene (curli biogenesis activator) and 77.75% carried csgA gene (curli biogenesis sununit)"CsgA and CsgB", are a structural curli fimbriae subunit proteins, which the Most diverse of them in Fecal *E. sakazakii* with a homology 78% with other enterobacteriaceae species[18],Zhou and coauthors, 2012 [19] come to conclusion that there is a match between curli subunit *E. coli*, *Salmonellae typhimuerium* LT2, And *Citrobacter koseri* were exhibited to cross cultured in vitro In the event that there are genetic mutations for one of the curli components

By a study presented by [20] found that the csg-BA operon inll (78.6%) of *E. cloaceae* isolates and the possibility of *E. cloaecae* to form biofilms was confidently linked with the mRNA expression of csgA. The results demonstrated that the curli fimbriae play a role in the biofilm formation of *E. cloacae*

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Conclusion

The *E. cloacae* possession of curli biogenesis genes helps them produce extracellular matrix and develop biofilm, and this explains why patients do not respond to some of the prescribed treatments

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