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Genotypic Detection of rcsB, afaB, fim Hgenes Coding Biofilm Production of Escherichia Coli Isolated from Pressure Ulcer

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Abstract--- *Pressure ulcer or bed ulcer are localized injuries to the skin and or underlying tissue that commonlyhappen over a bony prominences, infected pressure ulcer pose a great risk when left untreated ,they can affected other body system .the most common bacterial type found in infected ulcer are Pseudomonas aeruginosa , Escherichia coli , Staphylococcus aureus and Staphylococcus heamolyticus. Therefore 82 swab were collected from patient suffering from bed ulcer and admitted to Al-Sader medical city, and Al- Hakim General Hospitalthrough the term fromNovember 2018 to February 2019. The specimens was refined in suitable media(Mac Conky agar , and nutrient agar).after incubation period ,the result revealed that 119 bacteria isolates were isolated and identified according to vitek-2 system ,57(47.9%) of isolated were gram positive and 62(52.1%)were gram negative ,the gram negative represent by 25 isolated of Escherichia coli ,12 Klebsiella pneumonia ,14 Pseudomonas aeruginosa ,9 isolatesProutus mirabilis and one isolated for each Sertiaficania, Pseudomonas floursens. Genetically, study for rcsB, afaB, fim H gene encoding biofilm formation that associated with pathogensity of Escherichia coli by using PCR technique. The result of the present study reported that fimH gene was found in 23(92%) isolates of Escherichia coli, afaBgene was found in 19(76%) of Escherichia coli isolates and rcsB gene that 20(80%) of Escherichia coli isolates.*

Keywords--- *Escherichia Coli, rcsB, fimH, afabgenes.*

I. INTRODUCTION

Pressure ulcers are centralize region of injury to the skin and they at most effect patients that request bed relief. They are caused via external impose, like shear, pressure, a combination of both, and oftentimes happen over bony prominence(1).Resolution of the Wound often damage via viruses, bacterial, fungi reproduction and the production of exudates that give rise to maceration of healthy layers skin (2). large parameter such as obesity, smoking,aging, and malnutrition can promote the expansion of skin chronic damage and damage healings methods (3).According to the phase of ulcer: non-blanch capable erythema of skin intact (Stage I), partial-thickness skin lack include the dermis or epidermis (Stage II), full-thickness skin lack that might expand to the fascia (Stage III) or during this one in to the structures deeper, like bone muscle, or structures joint(Stage IV) (4).The most frequent pathogens the isolated from bed ulcer are : coagulase-negative staphylococci, Enterobacteriaceae, S.aureus, Enterococcus sp. and Corynebacterium sp(5).Many complications of are occur with bed ulcer which include cellulitis, osteomyelitis, septicemia, limb amputation and death(6).Many studies revealed the bacterial associated with bed ulcer infection produce or have sever virulence factors facilitated invasion the tissue (7). Staphylococcus

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haemolyticus produces a number of putative virulence factors,exopolysaccharides layer (slime) it has been associated with sepsis,extracellular polysaccharides seem to be the most important factor and biofilm production,produce α or β hemolysin(8). Escherichia coli an abundance of virulence parametercounting,fimbriae or pili though there are evidences of presence of adhesions in the cell surface of bacteria(9).Several toxic has the capability to an alter the host cell signaling cascade and modulate inflammatory responses(10)

Secreting siderphores to iron uptake (11,12). In Escherichia colircsB that acts as a positive regulator of colonic acid synthesis havesignificantpartof formation biofilm (13)

II. MATERIALS AND METHODS

Specimens Collection and Bacterial Identification

Atotal of 82 specimens were collected from patients suffering from bed ulcerThose specimens were collected from patients (male and female) by taken swab from infection areaSpecimens were inoculated on three types from culture media, which included blood agar, mannitol salt agar and MacConkey agar, that considered as predominant enrich media, selective and differential media of the isolation, identification and purification of many types of bacteria. The plates was incubated at 370C of 24 hour. Then a single pure isolated colony was transferred to trypticase soya agar of the preservation and to transport other biochemical tests and viteck system that inveterate the isolates of identification.

DNA Extraction

Genomic DNA was extracted viautilizing a commercial extraction method (Favorgen, Taiwan).

Molecular Identification

Gel electrophoresis was utilizedof discoveryfor DNA via UV trans illuminator.The P.C.R assay was performed to detect the rcsB gene of Escherichia coliTable2.This primer was designed viaα DNA company, Canada as in Table 1.Amplified produces was confirmed utilizing 0.8% gel agarose electrophoresis to estimation the P.C.R products size.

The gel was stained by 4 μL, (Sigma, USA) ethidium bromide of 10mg/mL and it run at 70v of 1.5 hour. A single band was observed atthe desired position on ultraviolet light transilluminator (Cleaver, UK); bands were photographed utilizing gel documentation method (Cleaver, UK). A 100bp ladder (Korea, Bioneer) was utilized to measure the Mwut. of amplified products (14).

Table 1: Primers Utilized in this Study

Primer Type	**Target DNA**	**DNA sequence (5′–3′)**	**Product Size (bp)**	**References**
rcsB	*rcsB*	F:TTAGTCTTTATCTGCCGGACTTAAGGTCAC R:TGAGAGGACTTGCTAATGAACAATATGAACGTA	200	([36])
fimH	*fimH*	F:TGCAGAACGGATAAGCCGTG R:GCAGTCACCTGCCCTCCGGTA	508	([35])
afaB	*afaB*	F:GCTGGGCAGCAAACTGATAACTCTC R:CATCAAGCTGTTTGTTCGTCCGCCG	750	([37])

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Table 2: P.C.R Program of Primer rcsB that Apply in the Thermocycler

Gene	Primary Denaturation	Denaturation	Annealing	Extension	Final Ext.	Cycles
RcsB	72˚C for 5min	72˚C for 1min	58˚C for 30 sec	94˚C for 30 sec	94˚C for 1min	30
Fim H	72˚C for 5min	72˚C for 1min	58˚C for 30 sec	94˚C for 30 sec	94˚C for 1min	30
afaB	72˚C for 5min	72˚C for 1min	58˚C for 30 sec	94˚C for 30 sec	94˚C for 1min	30

III. RESULTS AND DISCUSSION

Phenotypic Detection of Biofilm Formation of Escherichia Coli BIOFILM Production

Christensen's tube system was utilized to investigate the production biofilm, the results of this method showed that all bacterial isolates of Escherichia colihad ability to biofilm formation (Fig. 1).

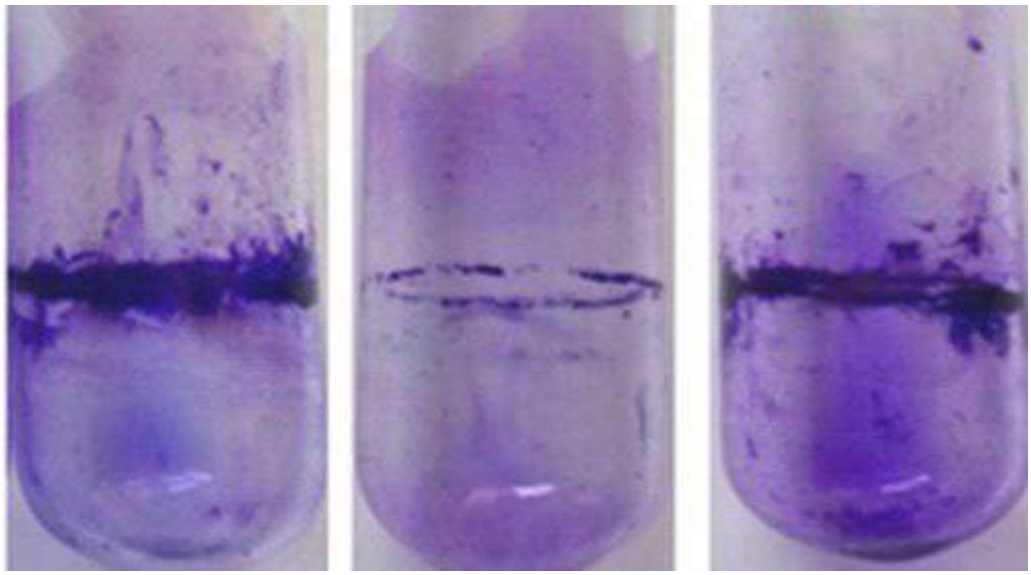

Figure 1: Phenotypic Detection Biofilm Production by Tube Adherence Test

Escherichia coli biofilm initiation and maturation can containseveral diverse parameter and its ability of biofilm depends considerably on ecological circumstances (15)

Even well-demonstrated adhesion parameter can be exchanged via anothers in exactly cases, numerous several pathways can be utilizedthrough E. coli formation of the biofilm, and deference regulatory mechanisms could coordinate the biofilm adhesion and maturation methods (16).In E. coli, motility has appear to need a link by its capability of a biofilm since its flagella allows bacteria to spread along the surface. Though, it is not a requirement, andnontitle bacteria can still of biofilms belowcertain conditions that the expression of robust adhesion parametermight substitute of force generating movements through the primary interactions amid adhering bacteria and the surface(17).Attached cells spread outward and upward by binary division to form cell clusters, or cells are recruited from the bulk fluid to form of biofilms, the relative contribution of thesemechanisms depends on the organisms, the nature of the surface, and the physical – chemical condition of the environment(18)The twitching

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motility, rate growth, cell signaling, exopolysaccharide production, and the physical growth environment wholly play a importantpart in the structure biofilm, bacteria growing in biofilms are responsible of great number of persistent infections and are often extra resistant to antibiotics than are free floating bacteria(19).Biofilms offer a mode of growth for bacteria that allow them to survive and indeed thrive in a host(20).Microorganisms by biofilm formation are associated throughseveral human infections (21)Biofilms can show an significantpart in protect bacteria of drugs exposure when compared throughanother bacteria don't have these virulence parameter, thus there was strong relationship amid resistance of prevalence and antibiotics of biofilms in bacteria (22)

Detection of rcsb, afaB and fim Hgene

Regulatory colonic acid synthesis (Rcs) order predominantly adjust genes interested in the production of significant cell surface-connected structures like LPS, flagella, fimbriae, exopolysaccharide, and ecological stress-related genes (23).The results of study revealed that 20(80%) of Escherichia coli isolates were carrying rcsB fig(2). Rcs method acts as a master repressor of biofilm formation via regulating the production of cyclic-di-GMP and biofilm matrix exopolysaccharide synthesis to facilitate ecological adaptation (23)Rcs activity also influences the formation of fimbriae in Escherichia coli by affecting the transcription of fim Band fim E(24-26).Improperly timed activation of Rcs impairs the functioning of other order desired of formation biofilm ,having expression for flagella(27).RcsF is anouter membrane lipoprotein that rotate stress signals to the searchlight kinase Rcs C. Recently synthesized RcsF is transported of an outer membrane (OM) β- barrel assembly complex via the periplasmic chaperone LolA, that assembled in to a college via OmpA(28).This step sequesters RcsF and prevents it of binding Iga.A, an IM protein that downregulates the Rcs pathway (28)

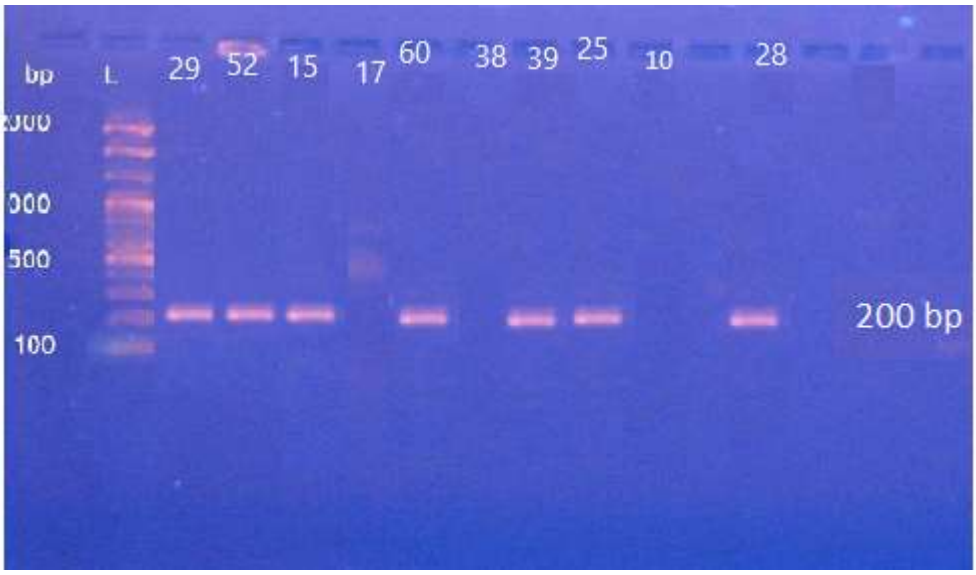

Figure 2: PCR Amplification for rcsbgene at Molecular Size=200 bp in Escherichia Coli: L-100bp DNA Ladder, Lanes Positive Isolates of Escherichia Coli in all Isolate Except (17,38 ,10)

The afa gene clusters encode fim bria adhesions (Afas) that are expressed by Escherichia coli strains, these gene clusters are responsible of the biosynthesis of the afa adhesions belonging to the afa/Dr family of adhesions and of

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the biosynthesis of invaseins. The genetic organization of the 6.7-kb DNA fragment encoding the AfaE-I adhesin involves five genes, afaA, afaE, afaD, afaB, and afaC(29) .

These five genes need localized and belong to the same transcription unit, The AfaB, AfaE and AfaC, gene produces are required for mannose-resistant hemagglutination (MRHA)(30) .

The data of the study shown that the afaBgene was find in 19(76%) of Escherichia coliisolates(fig.3).(31) find in their study that afaB gene was discovered in (85%) of Escherichia coli isolates ,but (32)found that (66.7%) of Escherichia coli transport afaB gene ,in adding(3) recorded in their study afaB gene was find in (40.5%) of Escherichia coliisolates .

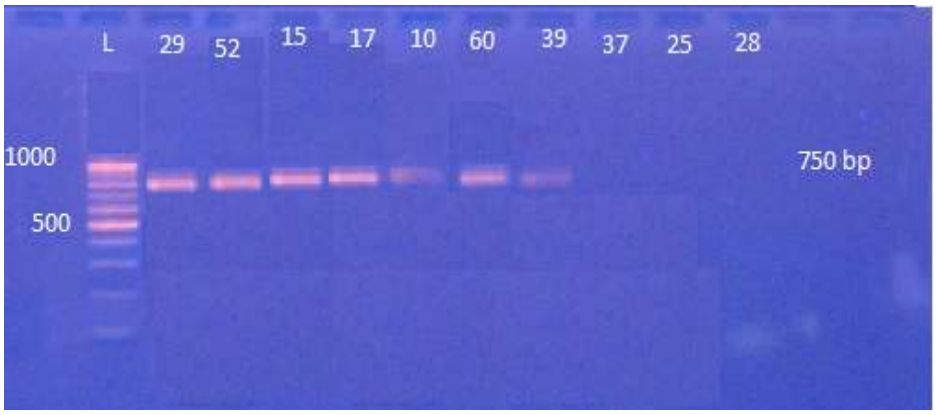

Figure 3: PCR Amplification for afab gene at Molecular Size=750 bp in Escherichia Coli: L-100bp DNA Ladder, Lanes Positive Isolates of Escherichia Coli in all Isolate Except (37,25,28)

The adhesive subunit of kind 1 fimbriae, encoded by Fim H, is a major determinant thatgreat tropism of urinary tract receptors; therefore, Fim H in colonizing Escherichia coliseveral niches (33). The result of the present study reported that fimH gene was found in 23(92%) isolates of Escherichia coli (fige4).The high prevalence of fimH indicates a certain role for such gene in adhesion.(34-36) they reported that (92%) and (93.33%), of UPEC isolates have fimH gene respectively.

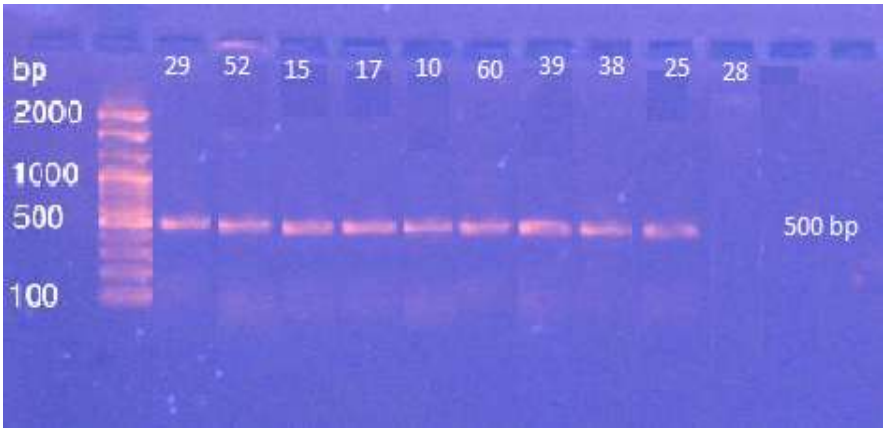

Figure 4: PCR Amplification for fim h gene at Molecular Size=500bp in E.Coli: L-2000 bp DNA Ladder, Lanes Positive in all Isolates of Escherichia Coli Except (28)

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IV. CONCLUSION

Study the for rcsB, afaB, fimH gene encoding biofilm formation that associated with pathogensity of Escherichia coli by using PCR technique. The result of the present study reported that fimH gene was found in 23(92%) isolates of Escherichia coli, afaBgene was found in 19(76%) of Escherichia coli isolates and rcsB gene that 20(80%) of Escherichia coli isolates.

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