WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 195-203. Research Article ISSN 2277– 7105

DETECTION OF VIRULENCE GENES(HLY,FIMH,KAPSMII) IN ESCHERICHIA COLI ISOLATES FROM BETA-THALASSEMIC AND NON THALASSEMIC PATIENTS BY USING PCR TECHINQUE

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Article Received on
26 May 2015,

Revised on 21 June 2015,
Accepted on 14 July 2015

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ABSTRACT

Background: Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. Beta Thalassemia, caused by a decrease in the production of β-globin chains, affects multiple organs and associated with considerable morbidity and mortality. This study aimed to comparsion between thalasemic and non thalasemic by used specific primers and identifying different microorganisms (aerobicand anaerobic) from urine and blood to detect. **Materials and methods:** The study samples consist of[41] Thalasemic and[18] non Thalasemic patients respectly. Isolation from mid urine and[25] Thalasmic and [25] non Thalasemic patients respectly. Isolation from Blood. **Results:** The main bacterial isolates were identified as47 isolates of *E.coli* (43.1 %), 9 isolates (8.3%) of S. Pneumonia., was 8 isolates of C. Pneumonia (7.3 %), 3 isolates of porteus and K. Pneumonia1 (11.9%). Whereas 6 isolates of Enterobacter colaca represented (5.5%), While P. aeruginosa represente 9 (8.3 %) and lastly Salmonella typhi and pseudomonas .spp(3.7%) for each In addition, Pantoea spp represents (2.8%). A highly significant difference was found between thalassaemic patients and non thalasemic found virulence genes. Bacterial in urine and blood of were found to be higher in the study compared to control group and the difference was statistically highly significant($P<0.01$). Molecular methods (PCR analysis)of virulence genes, resistance, genotyping of *E.coli* by using primers specific to type 1 fimbria gene (fimH) and capsule gene (kpsMTII), hemolysin (hly). The PCR assay results identified 45 fimH (58%) and kpsMTII (40%) while hly (28%). **Conclusion:** Patients with Beta thalassemic major had more infectionsseverity compared to non Thalasemicpitients.

KEYWORDS: β-thalassemia, Escherichia coli.

INTRODUCTION

Beta thalassemias are a group of inherited blood disorders caused by reduced or absent synthesis of the beta chains of hemoglobin.[1,2] From the few studies regarding bacterial infections isolation from urine and blood among patients with bacterima and UTI it was concluded that the prevalence and severity of this disease were higher in those patient than non thalasemic subjects.[3] There are not gentics studies of bacteria isolation from thalasemia and non thalasemia patients. This frist study was designed to compared between thalasemic and Non thalasemic patients by used specific pirmers.

MATERIAL AND METHODES

The study group included 109, with an age they were already diagnosed with β- thalassemia major, attending the thalassemic center in Ibn Al-Baladi Hospital for their regular checkup and blood transfusion. With the study group; Each individual was collecte blood and urine samples were suffering UTI and Bacterimia respectlly and DNA Extraction E. coli isolates were grown in Luria broth (LB, Himedia, India) then at 37 ºC overnight bacterial pelleted from broth and DNA extraction was done for 40 isolates.[4,5]

PCR detection of hly specific gene sequence (1117 bp product) was performed with primershly 1(/5AACAAGGATAAGCACTGTTCTGGCT3\) and hly 2(/5TCCATATAAGC GGTCATTCCCGTCA 3\) in total volume 25µl containing 2µl of template DNA (50 µg/ml), 2.5µl of PCR buffer (1X), 1.15 µl of MgCl2 (1.5mM), 0.5 µl dNTPs(200 mM), 3 µl of each primer (30 picamole) and 0.2 µl of Taq polymerase(1U/reaction). (Midland/USA). Samples were subjected to 30 PCR cycle each one consists of denaturation at 95 ºC for 1min, annealing at 60 ºC for30 sec. (incrementing by 1 ºC after every five cycles to 65 ºC) and extension at 72 ºC for 3 min then final extension at 72 ºC for 7 min. PCR reaction mixture were electrophoresed on 2 % agarose.[8]

PCR detection of fim Hspecific gene sequence (509bp product) was performed with Primers FimH forward (/5 TGC AGA ACG GAT AAG CCG TGG 3\) and fim H Reverse (/5 GCA GTC ACC TGC CCT CCG GTA 3\) in total volume 25µl containing 2µl of template DNA (50 µg/ml), 2.5µl of PCR buffer (1X), 1.15 µl of MgCl2 (1.5mM), 0.5µl dNTPs(200 mM), 3 µl of each primer (30 picamole) and 0.2µl of Taq polymerase(1U/reaction). (Midland/USA). Samples were subjected to 30 PCR cycle each one consists of denaturation at 95 ºC for 1min , annealing at 60 ºC for30 sec. (incrementing by 1 ºC after every five cycles to 65 ºC) and

extension at 72 ºC for 3 min then final extension at 72 ºC for 7 min. PCR reaction mixture were electrophoresed on 2 % agarose.[7]

PCR detection of kapsM specific gene sequence (272bp product) was performed with Primers forward (/5 GCG CAT TTG CTG ATA CTG TTG3'\)andkapsM Revese (/5 CAT CAG ACG ATA AGC ATG AGC A 3\) in total volume 25 µl containing 2 µl of template DNA (50 µg/ml), 2.5 µl of PCR buffer (1X), 1.15 µl of MgCl2 (1.5mM), 0.5 µl dNTPs(200 mM), 3 µl of each primer (30 picamole) and 0.2 µl of Taq polymerase(1U/reaction). (Midland/USA). Samples were subjected to 30 PCR cycle each one consists of denaturation at 95 ºC for 1min, annealing at 60 ºC for30 sec. (incrementing by 1 ºC after every five cycles to 65 ºC) and extension at 72 ºC for 3 min then final extension at 72 ºC for 7 min. PCR reaction mixture were electrophoresed on 2 % agarose.[7]

RESULTS AND DISCUSSION

41 and 18 bacterial isolates collected from thalasemic and non thalasemic patients as midstream urine samples of complicated UTIs and 25,25 blood samples were suffering bactermia and were identified by using cultural, morphological and biochemical tests. α-hemolysin is considered to be the most common type of hemolysin produced by UPEC that cause UTIs and their recurrence.[9,15] Results showed that 6isolates E1, E2, E3,E4 ,E6and E11 gave positive from thalassam iae and5 isolates E1, E3, E6,E7andE9 from non- thalassam iae encoded hemolysin gene as seen in following.

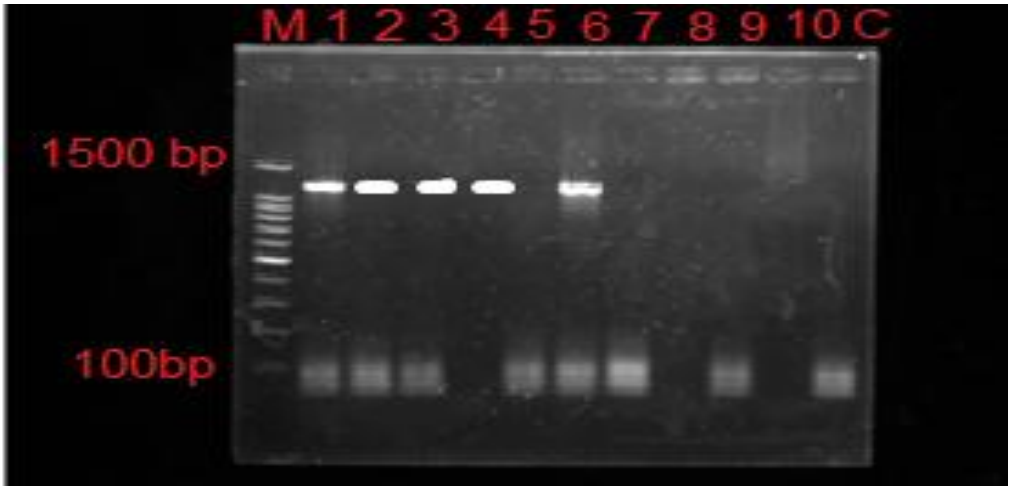

Fig. 1: Electrophoresis of 5 isolates of *E.coli* from healthy for detection of hly gene encoded for α-hemolysin. (agarose gel 2 %, 60 volt, 2 hours1-Line M : DNA marker (100-1500bp) 2-Line C is negative control.)

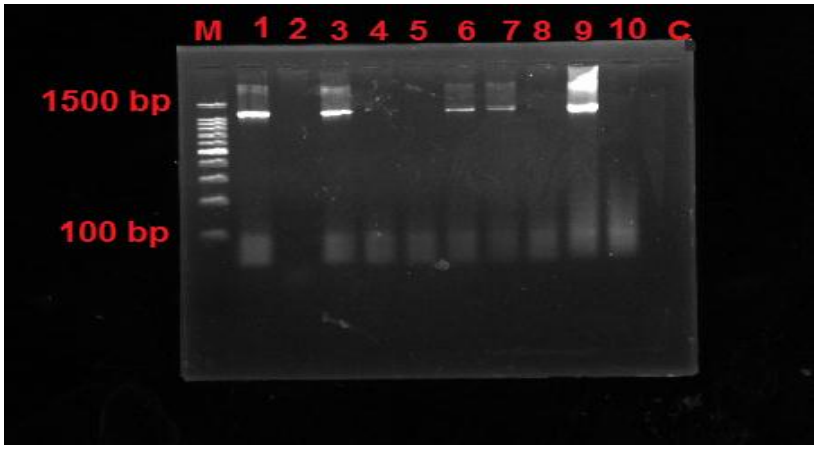

Fig. 2: Electrophoresis of 5 isolates of *E.coli* from healthy for detection of hly gene encoded for α-hemolysin. (agarose gel 2 %, 60 volt, 2 hours) 1-Line M : DNA marker (100-1500bp). 2-Line E1 is negative control.

Our results illustrated in Fig (3) showed that 44 (57%) isolates of *E.coli* produced hemolysin, while 33 (42.9%) isolates not produce hemolysin.

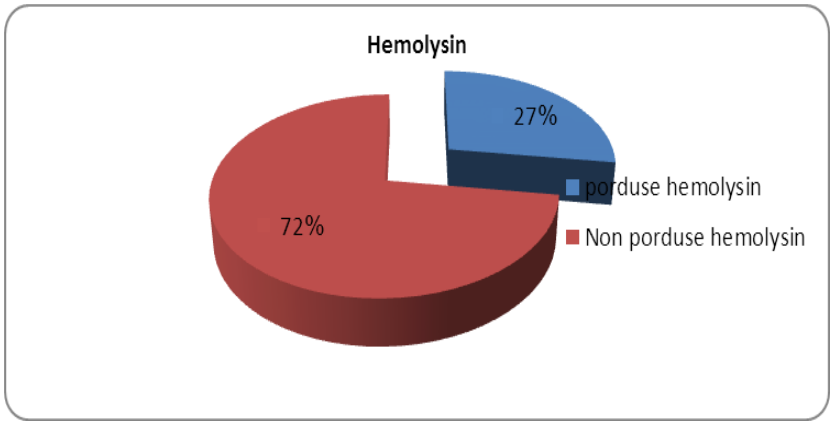

Fig. 3: Percentage of hemolysin producing *E.coli* isolates

This result showed found that the percentage of hemolysin production by E coli was (25.8%). Whereas the percentage of non hemolysin producing Ecoli is (72%) thatSeveral reasons explain the differences in the percentage by E coli such as source of blood, type of hemolysin was produced, source of bacteria and method to screen the production ability[7] and carried on chromosome or plasmid.[13]

Table 1: Distribution of sample study in Thalassemia and healthy according to hly gene

Group	Positive		Negative		Chi-square
	No.	%	No.	%	
Thalassemia (No. =20)	6	31.58	13	68.42	9.741 **
Healthy (No. =20)	5	25.00	15	75.00	
** (P<0.01) significant.					

The Statistical Analysis show thalassaemic patients is hight significant than healthy Patients who are splenectomised are often referred to as being immunocompromised.[17,18] This is because splenic macrophages are responsible for the filter and phagocytosis of bacterial and blood borne pathogens. Regular blood transfusion also results in immunomodulation. Hypersplenism is an absolute indication for splenectomy in thalassaemia major patientS.[19] that infections are more frequent or severe in patients with iron overload either related to genetic haemochromatosis or to transfusions, as in thalassaemiae,[20,21] fimH the adhesion factor Type 1 fimbriae is the most important factor which play a major role in the colonization of E. coli in different tissues in human body.[22] And 10 isolates (E1,E2,E3,E4,E5,E6.E7,E8.E9,E10) from thalasemaeiae and(E1,E3,E6,E9,E10) from non-thalasemic patients encoded fimH gave positive result and size of amplified band occur between (100–1500 bp) as shown in Fig(3,4). FimH adhesion was the most common virulence factor detected, having occurred in 23 (57.0%) of isolates.[13,16] These different of percentage. belong to fimh that is carried on a plasmid therefore not occurred on agrose gel after electrophores may be bacteria form urine and blood lost plasmid or genotyping of fimH found many types genotyping in the same starin of EcolI.[11,13]

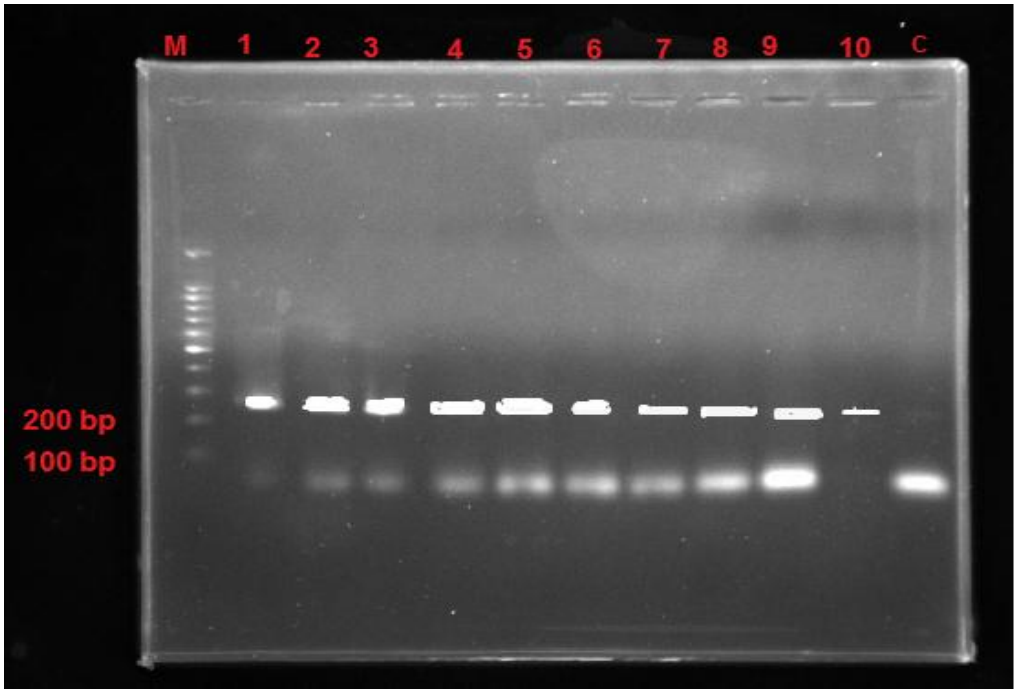

Figure 3: Agarose gel electrophoresis of PCR amplification products of *E.coli* fimH gene from thalassamaemiae (agarose gel 1.5%, 60 volt, 2 hours) 1-Line M : DNA marker (100-1500bp) 2-Line C is negative control.

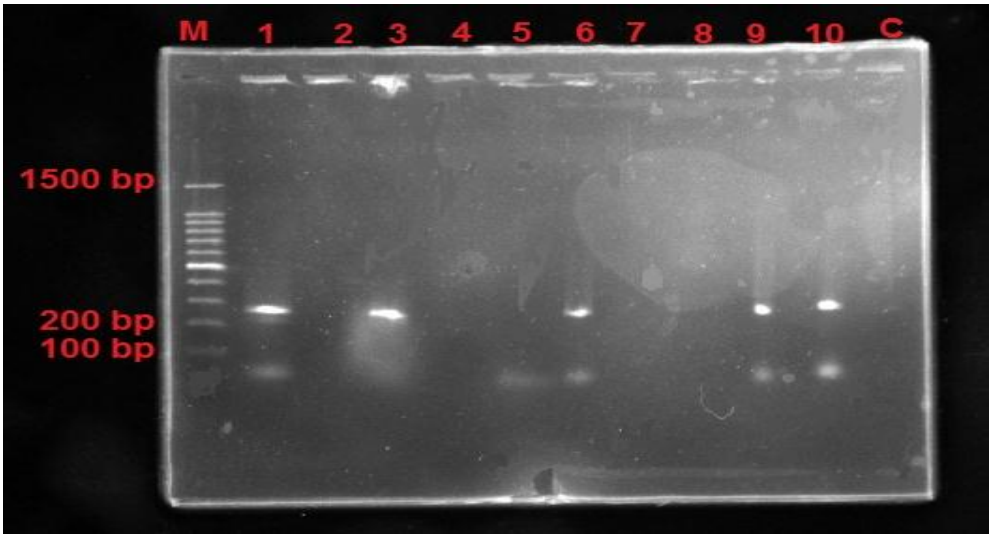

Figure 4: Agarose gel electrophoresis of PCR amplification products of *E.coli* fimH gene from healthy(agarose gel 1.5 %, 60 volt, 2 hours) 2-Line C is negative control 1-Line M : DNA marker (100-1500bp).

Table 2: Distribution of sample study in Thalassemia and healthy according to fim gene

Group	Positive		Negative		Chi-square
	No.	%	No.	%	
Thalassemia (No. =20)	15	37.5	5	25.00	6.018 **
Healthy (No. =20)	8	40.00	12	60.00	
** (P<0.01) significant.					

The Statistical Analysis show thalassaemic patients is hight significant than healthy Patients the reasons as same above in table 1.

kpsMTII gene present group II capsules determined by kps operon Capsule is common in Ecoli and is better known for contributing with urinary tract infections, bacteria need this virulence factor which helps the organisms to avoid[10,12,16] 15 (E1,E2,E3,E4,E5,E6,E9,E10.E11,E12,E13,E14,E15,E16,E17). Isolates gave positive results form thalaseamae and9 isolated(E1,.E3,E8,E9,E10,E12,E13,E14) Form healthy encoded kpsMTII size of amplified band occur (207 bp) as shown in Fig (5,6).

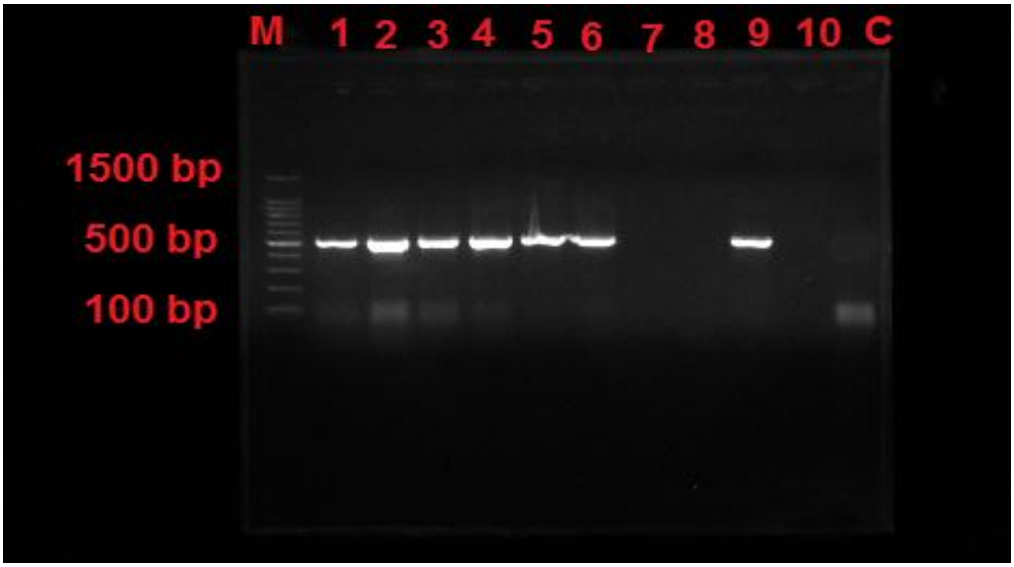

Figure (5): Agarose gel electrophoresis of PCR amplification products of E.col kpsMTII genefrom thalassamiae(agarose gel 1.5 %, 60 volt, 2 hours). 1-Line M: DNA marker (100-1500bp). 2-Line C is negative control

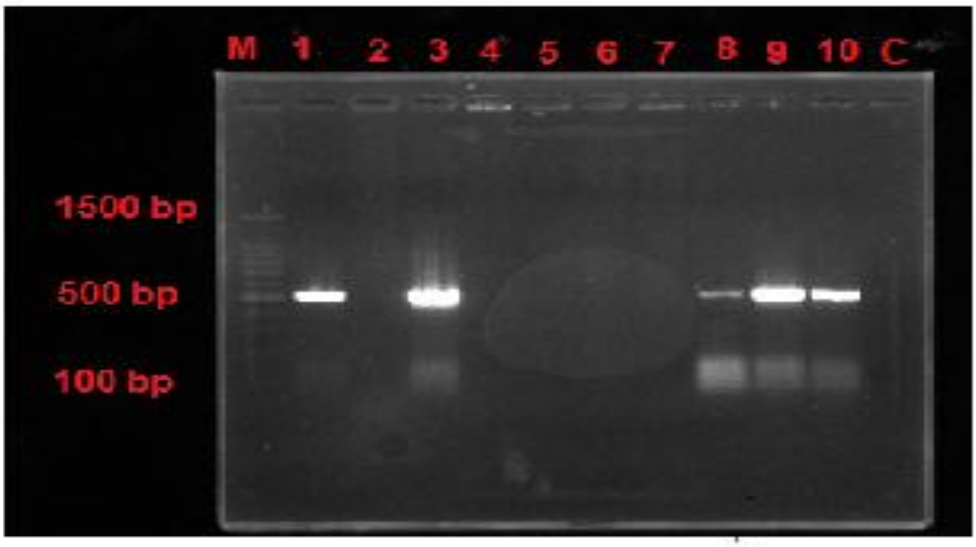

Figure (6): Agarose gel electrophoresis of PCR amplification products of E.col kpsMTII gene from healthy(agarose gel 2 %, 60 volt, 2 hours). 1-Line M: DNA marker (100-1500bp). 2-Line C is negative control

Table 3: Distribution of sample study in Thalassemia and healthy according to kpsMTII gene

	No.	%	No.	%	
Thalassemia (No. =20)	**14**	**73.68**	**6**	**30**	**12.375****
Healthy (No. =20)	**9**	**45.00**	**11**	**55.00**	
*** (P<0.05), ** (P<0.01).**					

The Statistical Analysis show thalassaemic patients is hight significant than healthy Patients the reasons as same above in table (1).

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