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RESEARCH ARTICLE

Detection of *Fima* and *Fimh* Genes in *Klebsiella pneumoniae* Isolated from Necrotizing Enterocolitis Patients in Infants

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Abstract

Sixty-four clinical isolates were collected from the children's protection hospital by Medical city in Baghdad for children infected with Necrotizing Enterocolitis where included (41) samples belong to stool and (14) samples belong to blood and (9) samples belong to urine for the period of 29/1/2018 to 4/4/2018. All the samples were cultured on MacConkey agar and Blood agar for the purpose of diagnosis. All isolates were identification depending on macroscopic, microscopic, biochemical tests and definite with Vitek-2 compact system, forty three isolates were obtained from all samples, (26) isolates as *K. pneumoniae* (60.46%) and 8 isolates as *E. coli* (18.60%) and 4 isolates as *P.aeruginosa* (9.30%) and 2 isolates were *K.oxytoca* (4.65%) and 2 isolates were *E. cloacae* (4.65%) and 1 isolates was *P. hauseri* (2.32%). The results showed that *K. pneumoniae* was the predominant in the samples which taken from infants infected to Necrotizing Enterocolitis. In these study, oame of virulence genes have been investigated for *K.pneumoniae* included both *fim*A and *fim*H gens which encodes for the fimbriae adhesion protein that belonging to type 1 fimbriae named Fim protein, and the results showed presence of *fim*A and *fim*H genes (100 %).

Keywords: *Klebsiella pneumoniae, Isolation, Identification, Necrotizing, Enterocoliti, fimA, fimH.*

Introduction

Klebsiella pneumoniae is a Gram-negative straight rods, arranged singly, in pairs or short chains, surrounded by a capsule, lactose fermenting, facultative anaerobic, nonmotile, having both a respiratory and a fermentative type of metabolism, characterized *K.pneumoniae* as oxidase negative, indol and methyl red; catalase positive [1,2]. *K.pneumoniae* is present widely spread in nature such as soil, water, plants and it exists in the normal flora of the mouth, skin and intestine [3]. As well as their presence on the mammalian mucosal surfaces such as a human, horses and pigs [4].

Among of the main reasons for the spread of *K. pneumoniae* in hospitals presented in its stability in the gastrointestinal tract of patients who are in hospital and intensive care units and the hands of workers in the intensive care and health, as well as medical devices which are reservoir for bacteria [5]. Besides to frequent use of medical devices which are in direct contact with body fluids among the patients who are in the hospital which leads to increase the injuries which resulted by making *K.pneumoniae* in form biofilm on those medical devices to promote antibiotic resistance [6]. *K.pneumoniae* is an opportunistic pathogen as it prepares a major cause of nosocomial pneumonia, septicaemia and urinary tract infections especially in newborns, blood cancer patients, immunocompromised candidates and diabetics' .Despite usage of appropriate antibiotic therapy, morbidity and mortality due to *Klebsiella* bacteraemia and pneumonia, more than 50% [7, 8].

Also *K.pneumoniae* causes pyogenic liver abscess, meningitis and necrotizing fasciitis. *K. pneumoniae* utilizes a many of virulence factors such as capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for

iron acquisition for survival and immune evasion during infection [9]. *K.pneumoniae* have at least three types of fimbriae; type 1 fimbriae, type 3 fimbriae and Kp (a-g) fimbriae [10,11]. *K.pneumoniae* has at least 78 capsular serotypes (K antigen) and prepares serotypes K1 and K2 more virulent patterns [9]. Characterized *K.pneumoniae* bacteria to its have 9 groups for O-antigen are (O1, O2, O2ac, O3, O4, O5, O7, O8 and O12), O1 is the most common serotype between clinical *K.pneumoniae* isolates [12], and plays O-antigen role in the protection of *K.pneumoniae* from arrival of the complement and thus the bacterial resistance complement mediated killing there are virulence factors own it *K. pneumoniae* such as iron acquisition which are considered necessary for bacterial growth in the body of the organism, as there are at least 12 iron absorption systems special in *K.pneumoniae*, produce *K.pneumoniae* a typical iron acquisition called enterobactin, which has the highest iron affinity [9].

Biofilm is one of virulent factors of *K.pneumoniae* and represented of biofilm to assembly and adhesion of bacterial cells on biotic and abiotic surfaces produced thick layer of extracellular biofilm which work on protection of the cells from phagocytosis by the immune system of host and remove effect of epithelial cells and antibiotics as well as determine of genetic diversity [13,14].

Necrotizing Enterocolitis (NEC) is disease infect of digestive system and it mainly occurs in preterm infants whith less than 37 weeks and their weights very few (less than 1500 gm) [15]. For the bacteria important role in occur NEC disease such as anaerobic bacteria and gram-negative bacteria and as well as occurs with cause weakness of immunity and viral infections and not complete of intestinal mature [16]. Alcantar-Curiel *et al.* pointed that the adhesion of *K.pneumoniae* to the epithelial cells due to her possess type 1 fimbriae and which encoded to gene called *fim* [20], and which has a role in the colonization of the urinary tract and inflammation [21].

Materials and Methods

Sixty four of clinical samples were collected from the children's protection hospital of the medical city in Baghdad for children infected with Necrotizing Enterocolitis, the clinical specimens included (41) stool, (14) Blood and (9) urine for the period of December 2018 to April 2018. All specimens were cultured on MacConkey agar and Blood agar then incubated aerobically for 24 hr at 37 ºC, Bacterial Isolates identification by Macroscopic, Microscopic and biochemical tests that include oxidase test, catalase, indol, methyl red, Voges-proskauer, citrate utilization, sugar test on kligler iron agar and urease test and definite with Vitek-2 compact system GN-card.

DNA was extracted according to the instructions of Promega Company; prepare the polymerase chain reaction mixture and a final volume of 20 microliter as following:

- 1 microliter from the forward primer of the gene.
- 1 microliter from the reverse primer of the gene.
- 2 microliter from DNA template.
- 6 microliter from deionized nuclease free water.
- 10 microliter from Go Taq® Green Master Mix 2X.

Then use a PCR device and adjust the optimal conditions for the polymerase chain reaction for detection of *fim*A gene and *fim*H gene, as shown in the Table (1). And The PCR product was detected using agarose electrophoresis with concentration of 1%.

Table 1: The optimal conditions for detection of *fim*A and *fim*H genes

No	Stage	Temperature	Time	Number of cycle
1	Initial DNA denaturation	95 ºC	5 min	1
2	DNA denaturation	95 ºC	30 sec	35
3	Annealing	62 ºC	30 sec	
4	Extension	72 ºC	45 sec	
5	Final extension	72 ºC	7 min	1

Results and Discussion

Sixty four clinical isolates were collected from the children's protection hospital by Medical city in Baghdad for children infected with Necrotizing Enterocolitis where included (41) samples belong to stool and (14) samples belong to blood and (9) samples belong to urine for the period of 29/1/2018 to 4/4/2018. All the samples were cultured on MacConkey agar and Blood agar for purpose diagnosis.

All bacterial isolates which grown on Blood agar grayish or white colonies whereas bacterial isolates which grown on MacConkey agar pink colonies and lactose fermenting colonies and other isolates showed pale colonies this non fermented to lactose. Below microscopic they reacted negatively and positively with Gram stain, its most were garm-negative bacilli and some of isolates were gram-positive. Shown results the biochemical tests to all bacterial isolates that the predominant bacteria was *Klebsiella pneumoniae*, its gave negative results to oxidase test, indol and methyl red, and positive to catalase test, citrate, voges-proskauer and urease, and glucose fermented and H2S was not created on kligler iron agar medium, while *Escherichia coli* gave negative result for oxidase test, citrate, voges-proskuer and urease and positive result to catalase test, indol, methyl red and H2S was not created. Either *Pseudomonas aeruginosa* showed negative result to indol test, and voges-proskauer, and positive result to oxidase test, catalase, citrate and variable result to methyl red and H2S was not created and variable to urease test. While *Klebsiella oxytoca* gave positive result to catalase test, indol, citrate, voges-proskauer and urease, and negative result to oxidase test and methyl red and glucose fermented and H2S was not created on KIA medium, Either *Enterobacter cloacae* showed positive result to catalase test, citrate, voges-proskauer and variable to urease test, and negative result to oxidase test and indol and methyl red and glucose fermented and H2S was not created on KIA medium. Whereas *Proteus hauseri* showed negative result to oxidase test, citrate and voges-proskauer, and positive result to catalase test, indol, methyl red and urease, glucose fermented and H_2S was not created on KIA medium. Use Vitek-2 system to diagnose the bacterial isolates taken from Stool , urine and blood for infants less than two years infected with Necrotizing Enterocolitis, its provide 64 test from biochemical test necessary to diagnose of bacterial isolates, after to determine the Macroscopic for colonies and the microscopic and conformist biochemical tests and definite with Vitek-2 system, 43 isolates were identified as gram-negative bacteria from 64 samples (stool. blood and urine) to infants, and the other samples were part of them Gram positive and other part did not show growth on blood agar and MacConkey agar, the number of isolates percentage of *K. pneumoniae* were 26 (60.46%) and *E.coli* 8 (18.60%) and *P.aeruginosa* 4(9.30%) and *K. oxytoca* 2 (4.65%) and *E.cloacae* 2 (4.65 %) and *Proteus hauseri* 1 (2.32%) as shown in Figure (1). And from during the results showed that the *K.pneumoniae* are the predominant species in the samples taken from infants infected with Necrotizing Enterocolitis and the number of isolates of *K. pneumoniae* bacteria isolated from the stool samples were 23 isolates (88.46%) and 2 isolates from blood (7.69%) and one isolate from urine (3.84%).

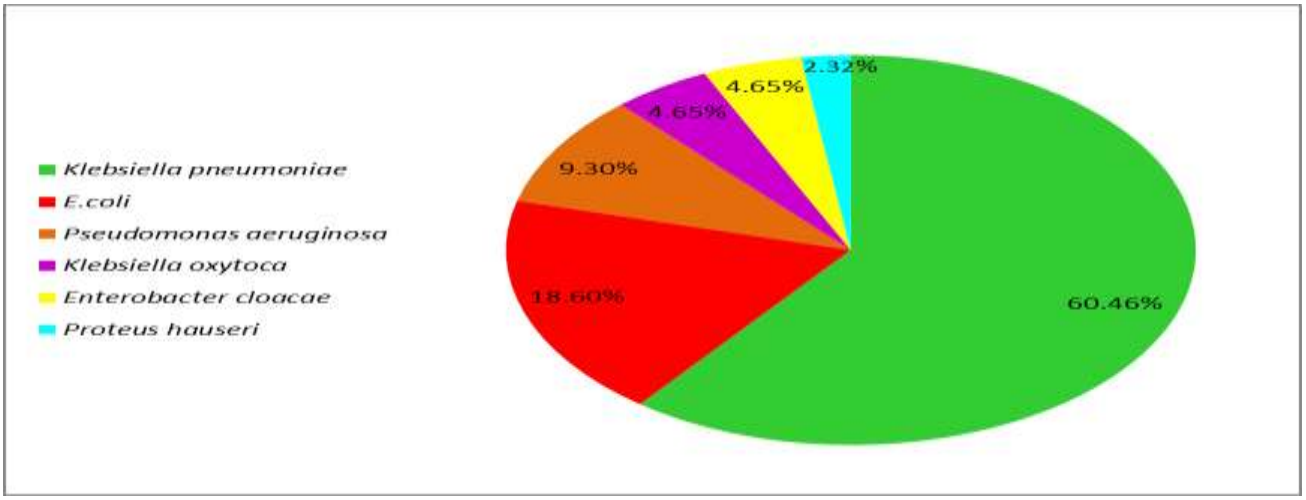

Figure 1: percentages for bacterial isolates which caused to Necrotizing Enterocolitis in infants

Study of Grishin *et al.* stated that *K. pneumoniae* bacteria recorded highest infection percentage to Necrotizing Enterocolitis disease in infants; either the other bacterial species causative to fewer percentages to Necrotizing Enterocolitis were *P.aeruginosa* and *Acinetobacter* and *Cronobacter sakazakii* [17]. While Warner *et al*. pointed that genera the Gram-negative bacillus bacteria caused Necrotizing Enterocolitis to variable percentages such as *Klebsiella* and *E.coli* and *Enterobacter* [18].

Whereas study of Raveh-Sadka *et al.* indicated that *K.oxytoca* caused Necrotizing Enterocolitis in infants to different percentages [19]. Total DNA was extracted from the clinical *K.pneumoniae* isolates taken from the stool samples to mostly and of blood, urine samples to very small percentages for preterm infants infected with Necrotizing Enterocolitis to using DNA extraction machines equipped by promega company, then the electrophoresis for DNA, the *fim*A and *fim*H genes were detected in the bacterial isolates under study to using the polymerase chain reaction method, the results revealed that the *K.pnumoniae* isolates have these the genes. After conducting the PCR of the DNA series to using special primers for the *K.pneumoniae* which target the specific sequence of genes under study for the purpose of detecting isolates that possess these genes, after conducting the interaction, then the electrophoresis for the PCR product on Agarose gel and after examing the agarose gel under the ultraviolet rays appeared one band at the same level for the *K.pneumoniae* isolates which possess these genes, this indicates the correlation of the primers with its complementary sequence in the DNA strand and the use of the ladder (100-1500) bp, where the results showed in the current study that they are equal in molecular weight for the resulting bands when compared to the ladder. The results showed that 28 isolates (clinical samples, most of which are stool samples for preterm infants with Necrotizing Enterocolitis) for *K.pneumoniae* has the *fim*A gene (100%), when compared DNA bands to the ladder found that the resulting bands with molecular weight (500bp) as in Figure (2).

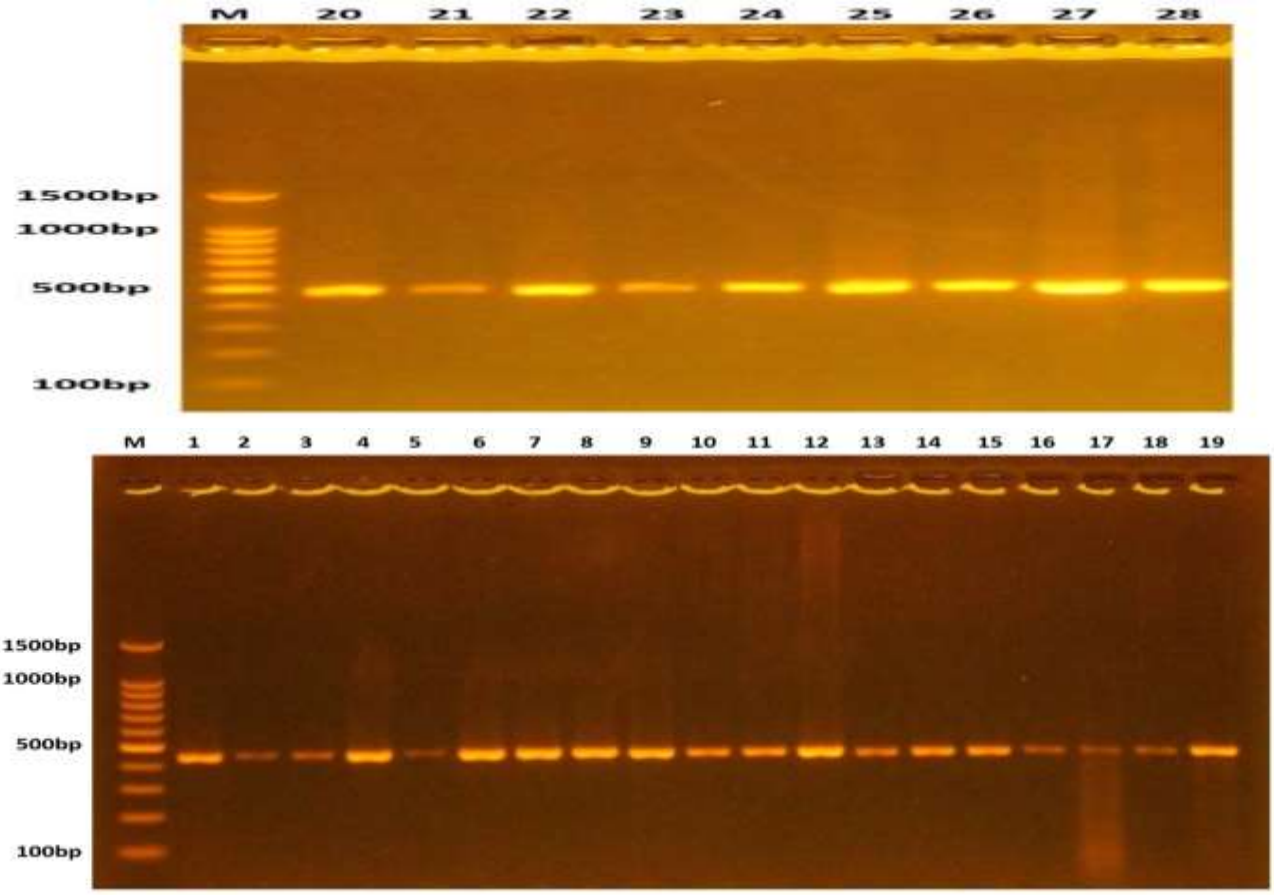

Figure 2: Electrophoresis of PCR product of *K.pneumoniae* isolated using a primer for *fim*A gene (500bp) using agarose gel (1%) with 100 volt for 75 minutes. Lane M: represent the ladder (100-1500) Bp, Lanes: (1-26) represent *fim*A gene amplification product for *K.pneumoniae* isolates isolated of stool samples, and (27-28) represent *K.pneumoniae* isolates isolated from blood and urine samples

Study of Alcantar – Curiel *et al* (2013), showed that (54) isolates of 69 isolates possessed the *fim*A gene (78%) referring at the same time the wide spread of operon *fim* in all of *K.pneumoniae* isolates [20]. Study of Li *et al*. and Klemm and Christiansen stated that *fim*A gene products are necessary for adhesion even in the absence of the main units of the fimbriae and which encoded her *fim*H gene [9, 22]. The results of the current study showed that 28 isolates belonging for the *K.pneumoniae* possessed the *fim*H gene (100%) and when compared the DNA bands to the ladder found that the resulting bands have a molecular weight (576 bp) as in Figure (3).

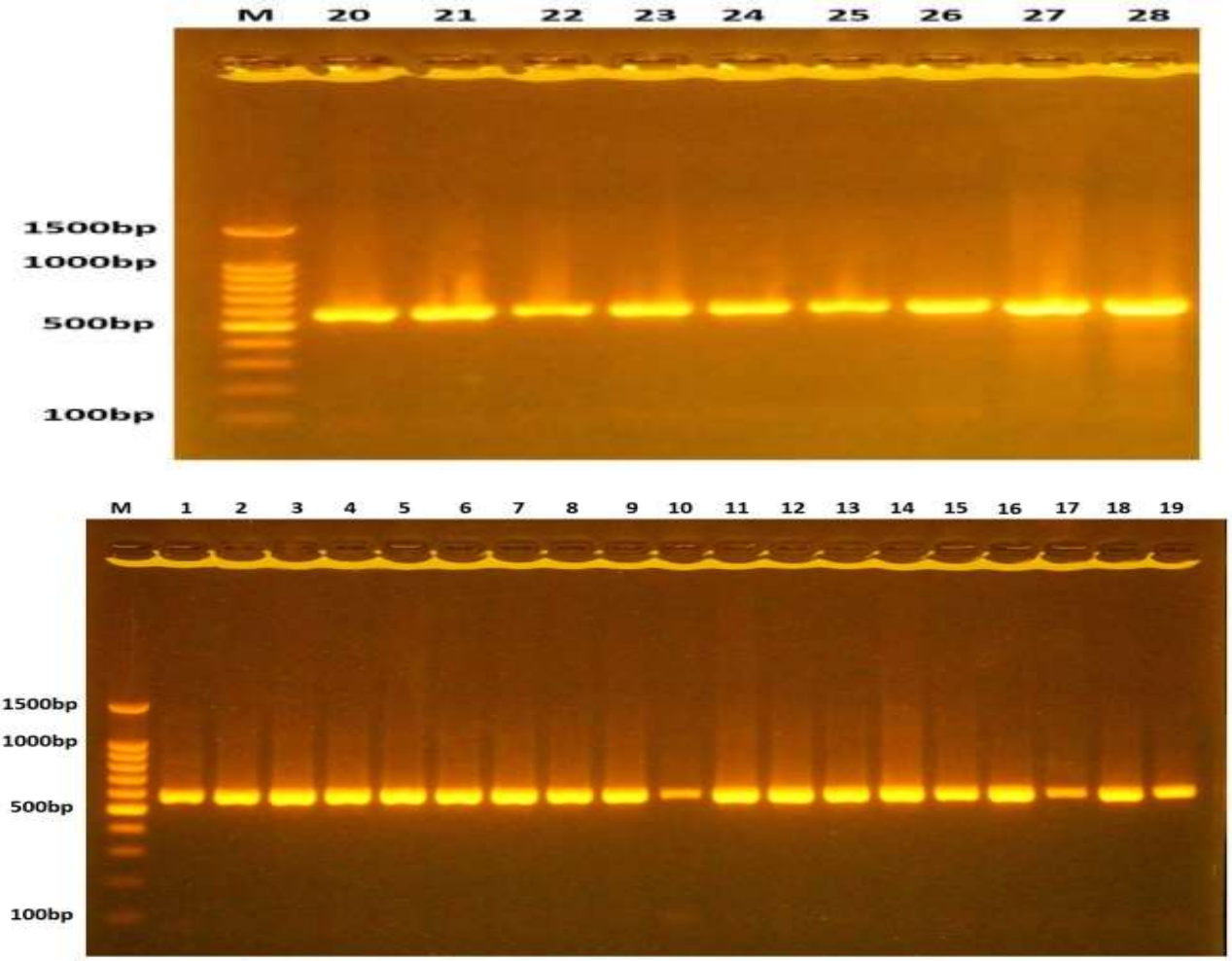

Figure 3: Electrophoresis of PCR product of *K.pneumoniae* isolates using a primer for *fim*H gene (576bp) using agarose gel (1%) with 100 volt for 75 minutes. Lane M: represent the ladder (100-1500) bp, Lanes: (1-26) represent *fim*H gene amplification product for *K.pneumoniae* isolates isolated of stool samples, and (27-28) represent *K.pneumoniae* isolates isolated from blood and urine samples

Study of Alcantar-Curiel et al (2013), indicated that K.pneumoniae isolates which do not possess fimA gene showed her possess fimH gene (100%) referring at the same time high presence fimH gene between K.pneumoniae isolates [20]. While Li stated that fim operon which encoded to type 1 fimbriae which works on K. pneumoniae adhesion in urinary tract tissues and not contributed those fimbriae in K.pneumoniae colonization for lung and intestine tissues [9]. The results showed that percentages the fimA and fimH genes in K.pneumoniae isolated from Necrotizing Enterocolitis patients were more than global studies and this due to the isolate source.

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