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# Molecular screening of biofilm formation by *klebsiella pneumoniae* isolated from children under 5years in Babylon province

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**ABSTRACT**

Background: Klebsiella pneumoniae is a major causative agent of diarrhea and acute enteritis in children under 5 years of age, which plays a major role in the development of harmful carcinogenic biofilms due to its strong colonization capacity. The various cell surface molecules and their various structures were the reason for the development and formation of biofilms of klebsiella pneumoniae . Many topics referred to as biofilms, are associated with many health problems. The ability of K.pnemoniae on the formation of the biofilm, as well as the role of a number of virulence factor genes on its formation and contributes to the stability and survival of K. pnemoniae infections. The biofilm is one of the virulence factors of K. pneumoniae. The membrane represents the bacterial cells aggregating and adhering to the living and non-living surfaces producing a thick layer of the extracellular biofilm that works to protect the cells from phagocytosis by the immune system of the host, eliminating the effect of the epithelial cells, and antibiotics. **Objectives**: to study the genes responsible for the formation of biofilm by klebsiella pneumoniae. **Material and Method**: the samples have collected from Children under 5years old from Women's Hospital for Childbirth , Al Noor Hospital for Children, and Al-Sadiq hospital in Hilla city during the period from September month 2020 to March 2021. The population of study was consist of 1hundred, 30 healthy individual apparently no disease is shown, while the other group are sick and include (80 patients had acute gastroenteritis, 48 of the females, 32 of the males. **Results :** according to the diagnosis of klebsiella pneumoniae isolates that is based on standard laboratory protocols and confirmation of the diagnosis with the VITEC system, our results showed 31.25% is a Klebsiella was the dominant type in the samples and this activity of Klebsiella was achieved to form biofilms by tube method. **Conclusion:** The results showed that the *Klebsiella* bacteria are the strongest in the biofilm formation this based on molecular genes in bacteria such as wbbm, wzm and Mrkd gene.
**Keyword:** *klebsiella pneumoniae, biofilm, wbbm and wzm.*

## Introduction

Acute gastroenteritis (AGE) is one of the most common infectious diseases in children, characterized by sudden onset of diarrhoea with or without vomiting. Gastroenteritis is transmitted from person to person or acquired by the consumption of tainted food or drink ("food poisoning"). Bacterial pathogens are commonly found in undercooked or improperly preserved cooked or processed meats (chicken, beef, pork) and seafood. [1]. Diarrhea and vomiting Infectious remain as the most common causes of death among children under five years of age; it is being the first or main reason of death [2] It is caused by infectious, bacterial, and parasitic organisms, and it has an age, host, and geographic pattern. The etiology is generally overlooked, and oral rehydration therapy is the standard treatment [3]. Specific clinical features, such as fever, abdominal pain, blood in the stool, and fecal leukocytes, can be associated with bacterial infections.[4]. The etiological pattern of bacteria that causes acute diarrhea differs depending on where you live. Each year, more than half a million babies and young children die in developing countries as a result of AGE, and Vibrio cholerae continues to cause epidemics [5]. Klebsiella pneumoniae is a successful opportunistic pathogen associated with various ailments such as urinary tract infections, septicaemia, respiratory tract infections and diarrhea [6]. A variety of virulence factors, especially fimbriae, antiphagocytic capsule (CPS), LPS, membrane transporters and

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siderophores, allow the survival of K. pneumoniae and its immune evasion during infection [7]. The ability of K. pneumoniae to form biofilm protects the pathogen from the host immune responses as well as from antibiotics[8]

## Materials and Methods

### Patients and Healthy control:

110 samples were collected from children suffering from diarrhea who were admitted from the Women and Children's Hospitals, Al-Sadiq Hospital and Al-Nour Hospital for Children in Hilla, in the months of September 2020 and March 2021 A specialized physician examined all of the acute gastroenteritis patients and diagnosed them as having acute gastroenteritis. Many of the mothers are questioned about their age, history of admission to the hospital for acute gastroenteritis, and other diseases. The research is explained to both the patients and the control group.

### Methods:

Klebsiella pneumoniae was identified according to standard microbiological and biochemical protocols and the VITEC system . The biofilm composition was studied by using the tube method. Determination of Klebsiella pneumoniae genotypes as following:

### Molecular Technique:

This technique was used to detect the klebsiella wbbm and wzm genes in patient and control samples; in this technique, DNA was extracted and the gene was amplified by PCR. The genes involved in biofilm formation were investigated using polymerase chain reaction and unique primers based on the wbbm and wzm genes. Klebsiella pneumoniae isolates were found in 31.60 percent of the samples. The wbbm and wzm genes responsible for biofilm formation were found in 31.60 percent of klebsiella pneumoniae isolates from infants with diarrhea.

**Table 1.** Specific Primers for (wzm and wbbm) genes used in this study.

| Gene | Primer sequence |
|---|---|
| Wbbm | F:ATGCGGGTGAGAACAAACCA<br>R:AGCCGCTAACGACATCTGAC |
| Wzm | F:TGCCAGTTCGGCCACTAAC<br>R:GACAACAATAACCGGATGG |

### Detection of biofilm formation:

The tube adherence approach was used to identify the biofilms. As in the case of [9]. The investigation of biofilm development was based on the biofilms' adherence to borosilicate test tubes. The tube method (biofilm assay) was used to detect K.p. biofilm formation and was considered a standard test for detecting biofilm formation using crystal violet.

### Statistical Analysis:

The data was examined using SPSS version 16 software (Statistical Package for Social Sciences). $P<0.05$ was used to determine statistical significance.

## Results and Discussion

After 4-10 hours of microorganism cultivation, the morphologies of biofilms were studied in vitro. Bacterial vegetative approaches were found to have reversible adhesion. The findings revealed that Klebsiella pneumoniae is the most effective at forming biofilm. Our findings were consistent with the findings of[10]. which found that K. pneumonia was a good biofilm producer., as shown in Figure (1). Figure (2) shows the quantitative results (mean± SD. OD594 nm) of crystal violet stain of biofilm made by *K. pneumonia*e. It showed an increase in biofilm formation, with a peak on the fifth day of incubation, after which the biofilm formation decreased gradually. The adhesive properties of microorganisms were used to identify their pathogenic properties, according to this report. High adhesive potential is one of the most significant factors in

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biofilm design, which is characterized by an increase in optical density and multiple drug resistance, according to previous studies [11] K. pneumoniae's ability to form a mixed-species biofilm in vitro can determine the abundance and spatial localization of each species within the biofilm. When compared to single-species biofilms, the production of mixed-species biofilms took 1–2 days longer. The increasing evidence of K. pneumonia's capacity to shape biofilm, primarily on medical devices, as well as recent data linking such actions to the acquisition of antibiotic resistance, may raise even more concern about the pathogen's threat in hospital settings.

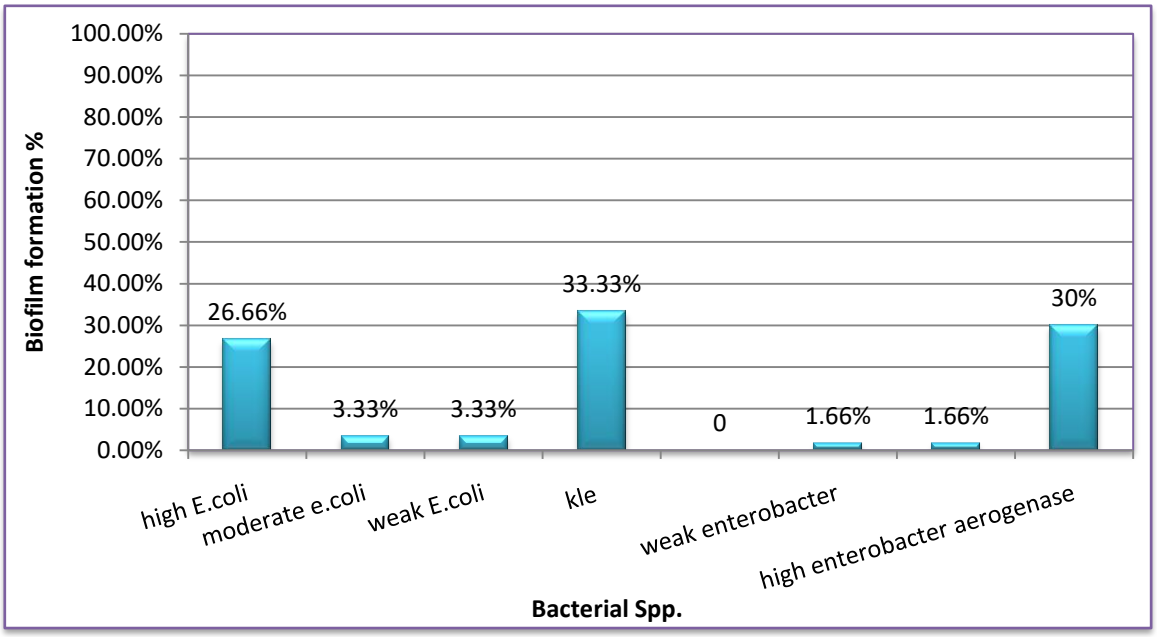


**Figure 1.** The difference in the biofilm formation ratios for the isolated bacterial species .

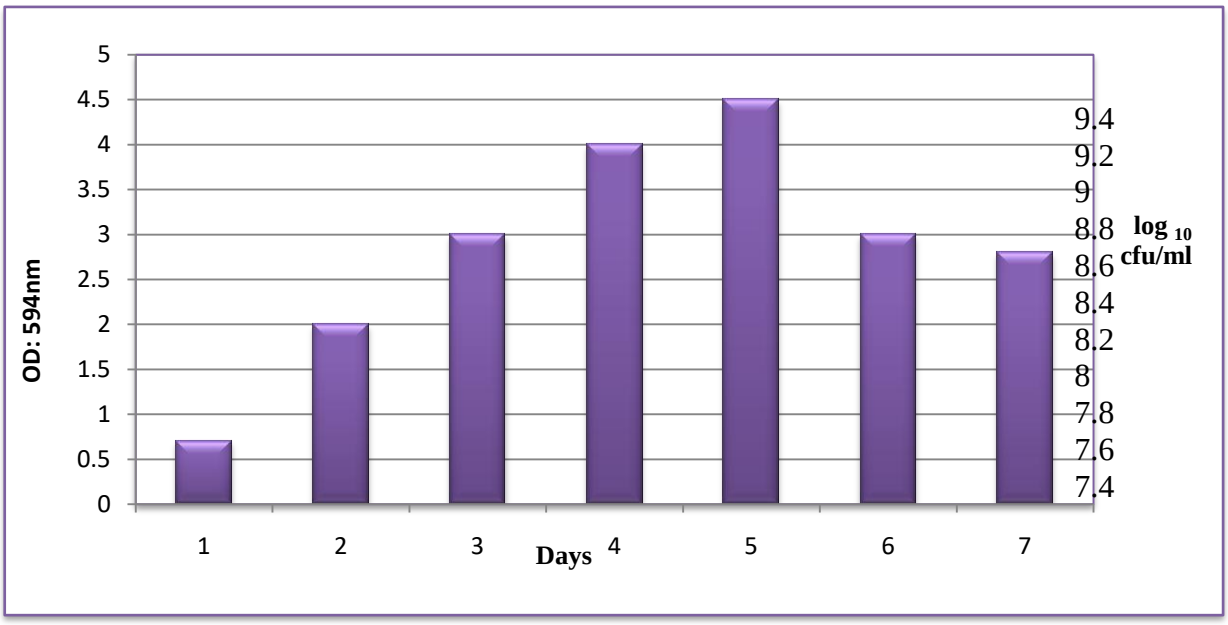


**Figure 2.** Biofilm formation by *K. pneumoniae* for 7 days as determined by crystal violet staining (OD: 594nm) . coefficient of variation (CV ).

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For The Molecular detection of *K. pneumoniae* genes, In this study revealed the wzm and wbbm genes in the bacterial isolates under study by using the Polymerase chain reaction (PCR) technique. Gel electrophoresis analysis using DNA marker 100bp length"(KAPA Universal Ladder)" revealed the products of active attachment between the extracted DNA and particular primers for wzm and wbbm genes promoter site, molecular typing is a useful technique.[12]. Our findings support [13]observation that pathogenic K. pneumoniae strains are extremely heterogeneous due to variations in nucleotide sequences. Figure (3) show the The wzm gene was used to detect the ability of the bacteria to adhesion. The inflation process of the DNA and the initiator of the wzm gene showed the positive result for all samples.

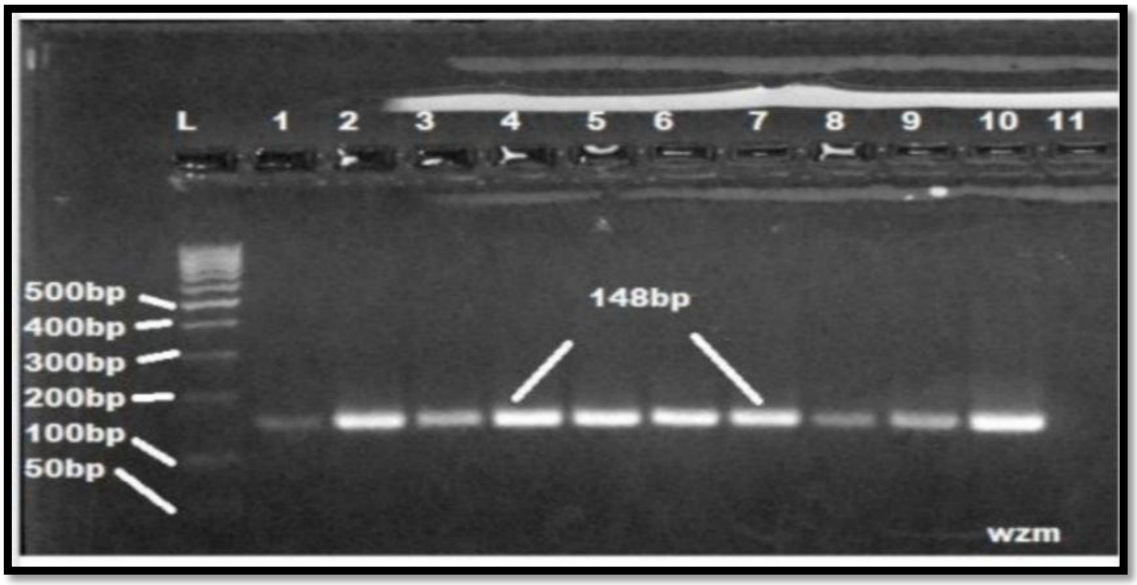


**Figure 3.** Ethidium bromide-stained agarose gel of PCR amplified *K. pneumonia* genes show:

- Lane (L):DNA molecular size marker (100 bp ladder).
- Lane 1 - 11 for wzm gene of *K. pneumoniae* isolates DNA

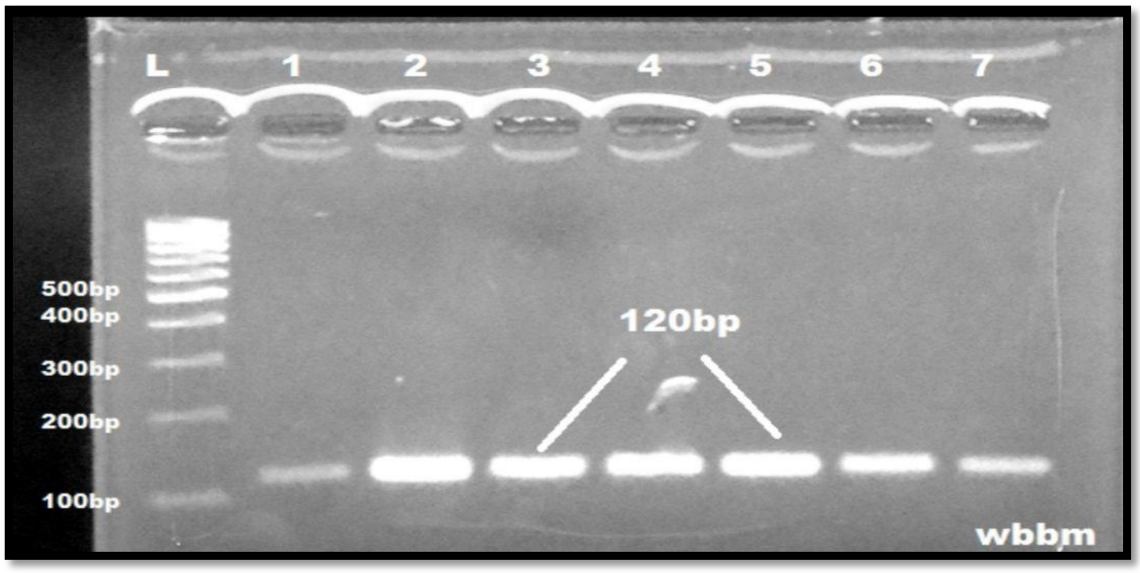

**Figure 4.** Ethidium bromide-stained agarose gel of PCR amplified *K. pneumoniae* genes show:

- Lane (L):DNA molecular size marker (100 bp ladder).
- Lane 1 – 7 for wbbm gene of *K. pneumoniae* isolates DNA

**Conclusion:**

This research reveals that the examined K. pneumoniae strains have a higher capacity to form biofilm, allowing researchers to speculate on the reasons for this. Thus, in addition to using adhesins and exopolysaccharides to develop in a sessile mode, they appear to use a variety of mechanisms to create a rich and complex biofilm, including active efflux, the QS system, and LPS development. These mechanisms, some of which are considered as important virulence factors (e.g. LPS) or relevant in antibiotic resistance.

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