# Molecular study of bacteria *Pantoea* spp. in Diwaniyah city,Iraq

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## Abstract

*Pantoea* spp. possesses many virulent genes that help to cause many human infections. Therefore, the present study was comprehensive of clinical and environmental samples to determine the extent of the spread of *Pantoea* spp. in the hospitals in the city of Diwaniyah.

A total of 623 samples were collected and distributed as483 samples of different clinical cases (77.52%) and 140 environmental samples (22.47%) from Diwaniyah city hospitals. The results showed that 24 isolates of *Pantoea* spp.s had been confirmed using API-20E Polymerase chain reaction technology.

All isolates of *Pantoea* spp were tested for the prevalence of virulence genes (*rcsB, hpaA, , hrc, rcsA*), using the polymerase chain reaction (PCR) technique. The highest incidence of *hrc* gene (70.83) .The presence of *rcsA* gene was 68.42% with 13 isolates. The current study did not record any presence of the virulence genes( *rcsB, hpaA*) among the isolates of *Pantoea* spp.

## Introduction

*Pantoea* spp. bacteria is known to belong to the enterobacteriaceae family, which is negative on Gram Stain (1).

The main problem is that the *Pantoea* spp. bacteria are associated with opportunistic diseases, although they are not a compulsory infection (2).

*Pantoea* spp. have *hrc, rcsA* genes that are responsible for the production of exopolysaccharide (EPS) were identified which help with the process of bacterial adhesion and the enzymes that have a role in the pathogenesis of these bacteria, such as Cellulase enzyme, which helps analyze the walls of plant cells consisting of a thick layer of cellulose polymer that provides protection of the plant from the penetration of microbes and increasing the ferocity of the bacteria causing the disease inside host cells (3) .

*rcsA* and *rcsB* genes are regulatory genes that control the production of capsule polysaccharide synthesis (EPS) (exopolysaccharide) (4).

The *hpaA* gene also increases the secretion of pilius and confuses host defense devices and increases virulence in them because pilius increases the susceptibility of bacteria to adhering to host tissue and thereby manipulating cellular host activity (5).

**Collection of Samples**

As many as 483 clinical and 140 environmental samples were collected from Diwaniyah hospitals including Diwaniyah General Teaching Hospital, Women's and Children Teaching Hospital Children, Burns Specialist and Consultancy Center, and the Public Health Laboratory for the period from October 2017 to June 2018. Swabs were collected from different clinical samples, which included 82 samples and swaps from pharynx (23), ears (49), dialysis (83), stool (solid and diarrhea) (27), pus (31) (27), wounds (44), burns (70) and cough (74). Environmental

samples include floor (30), medical tools (40), equipment (20), walls (30) and beds (20). Medical cotton swabs containing the center of the transport media swabs were used in sampling to ensure the vitality of the isolates, and then the necessary tests in vitro and biochemical laboratory of the Faculty of Science / University of Al-Qadisiyah for the bacteria *Pantoea* spp. were applied.

**DNA primers**

Gene 16rsRNA primers were used for the diagnosis of *Pantoea* spp. The primer genes responsible of identifying virulence genes in these bacteria were used. All primers were designed using NCBI-Genbank and primer3plus design. These primers were prepared by Bioneer Co. in Korea (Table 1).

**Table 1: DNA primers used in this study**

| Primer | | Sequence | Amplicon | Gene bank code |
|---|---|---|---|---|
| *16srRNA* | F | CCTGGACAAAGACTGACGCT | **523bp** | **FR832419.1** |
| | R | CGCTTCTCTTTGTATGCGCC | | |
| | R | TTCCATGATGCCGCTCACAT | | |
| *rcsA* | F | AAGTCCATCCGTTGACGCTT | **395bp** | **M60621.1** |
| | R | CAATTTACCGATGGCTGCCG | | |
| *rcsB* | F | GAACAACAACCCGGCCATTC | **438bp** | **Y09848.1** |
| | R | CTTTTGATAAGGCCGCCACG | | |
| *Hrc* | F | TACAGCCAGCTGATTGCCAA | **647bp** | **U56662.2:3505-5535** |
| | R | CGCTGTGAGATTTCATGGCG | | |

| | | | | |
|---|---|---|---|---|
| | R | AGATGGGCTGTGCGTCATC | | |
| | R | CAACACGATGCAAGAGACGC | | |
| *HpaA* | F | TGGGCAGTAACGATGTGCAT | **519bp** | **NC-003197.2:1195402-1196306** |
| | R | AAAGTTTCAGTTCACCGCGC | | |

Forword, **R:** Reverse **F**

**DNA-Amplifying PCR Thermocycler Programs**

Polymerization enzyme chain reaction was applied using PCR Thermocycler as in the following table.

**Table 2: temperature conditions used in the PCR**

| **Gene Name** | **Temperature (°C) / Time** | | | | | **Cycles Numbr** |
|---|---|---|---|---|---|---|
| | **Initial Denaturat-ion / time** | **Cycling conditions** | | | **Final Extension / time** | |
| | | **Denaturation** | **Annealing** | **Extension** | | |
| ***16srRNA hrc rcsA*** | **95/5 min** | **95/30Sec.** | **58/30Sec** | **72/1min** | **72 /5min** | **35** |
| ***rcsB*** | **95/5 min** | **95 /30Sec.** | **59/1 min** | **72/1 min** | **72/5min** | **35** |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***hpaA*** | **95/5min** | **95/30sec.** | **57/30sec** | **72/3min** | **72/5min** | **35** |

## Results and Discussions

## Diagnosis of *Pantoea* spp.using PCR technology

The isolates of *Pantoea* spp. in this study, have been diagnosed using the technique of polymerase chain reaction (Monoplex PCR) with 16S rRNA gene. The results in Figure 1 show that all isolates contain 16S rRNA gene with molecular weight (523 bp), which represents the diagnostic gene for these bacteria. This confirms that the isolates belong to Pantoea spp. genes. The PCR technique was 100% sensitive to the diagnosis of isolates during this study.

The study of (6) indicated that 16S rRNA gene was used to detect *Pantoea* spp. in 18 isolates confirming that 9 isolates belonged to this bacterial family. However, the study of (7) diagnosed *Pantoea* spp. isolates that are pathogenic to humans and animals using a 16S rRNA gene with 95.6% of its isolates containing this gene.

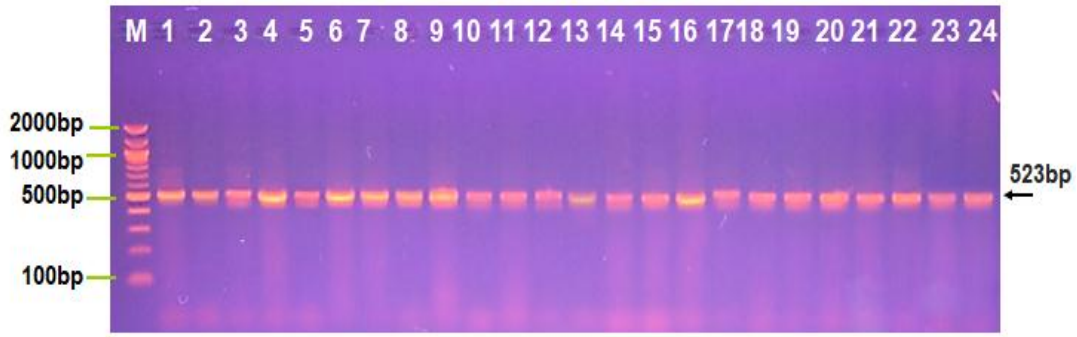


**Figure 1: Agarose gel electrophoresis of PCR assay show *16SrRNA* gene analysis of the *Pantoea* spp. The M: Marker ladder 2000-100bp and the trenching from 1-24 are the isolates of the positive *Pantoea* spp. with an 523bp and using an electric current of 80 amps and 100 volts for one hour.**

## Virulence Genes in *Pantoea* spp. Isolates

The detection of the presence of virulence genes in the isolates of *Pantoea* spp. is of great importance in epidemiology in Iraq in general because of the lack of local studies on the prevalence of virulent genes in the clinical isolates of the bacteria *Pantoea* spp., and in the hospitals of the city of Diwaniyah in particular since there have been no previous studies of the presence of isolates of these bacteria in hospitals with the spread of genes virulence in In this study.

Table 3 shows the distribution and spread of four genes (*hrc*, *rcsA*, *rcsB*, *hpaA*) in the isolates of *Pantoea* spp.

The results of the current study showed the presence of the *hrc* gene in 17 isolates of *Pantoea* spp. (%70.83). The number of *P. agglomerans* isolates containing this gene was 8 (33.33%) and as 9 for *P.calida* (37.5%) (Figure2). This result is consistent with those of (8) about the spread of this gene in *Pantoea* spp. isolates, especially in *P.agglomerans*

and *P.calida*. also, (9) recorded the presence of *hrc* in high quantities in *P. agglomerans* compared to other bacterial species belonging to the intestinal family.

As for the presence of *rcsA* gene in the isolates of the studied *Pantoea* spp., 68.42% (13 isolates) was recorded figure(3), *P. agglomerans* and *P.calida* recorded 25% and 29.16% (6,7 isolates)respectively .This is what was noted by (10) as the presence of *rcsA* gene in *Pantoea* spp. which is encoded to produce EPS as one of the factors of virulence in the pathogenic bacteria, which has a strong effect on the emergence of symptoms of the disease on the host as RcsA proteins encodes the gene to manufacture the capsule polysaccharides being important for the disease of bacteria. Sudies have indicated that the presence of gene *rcsA* and *rcsB* is crucial to the strains coexisting with the host to be converted to pathogenic strains and enhancing the formation of biofilm which also increases the virulence of pathogenic bacteria (11).

The current study did not record any presence of virulence genes (*rcsB, hpaA*,)figure (4,5) respectively in *Pantoea* spp. isolates (Table3). However. Also, (9) concluded that *P.agglomerans* bacteria contain *hpaA* gene. Studies have shown that the *hpaA* gene destroys host cells and immune devices and inhibits the host cells, which is contributing to increased virulence and disease progression (5). In other studies, the *hpaA* gene is affected by mutations, especially pathogenic bacteria, but it retains the stimulation of other pathogen-related genes. Also, studies pointed to the importance of HpaA proteins in the interference with the workings of host cells as these proteins are transferred to the host cell through the pathway in the period before the causing of severe disease symptoms (12).

**Table 3: Virulence Genes Distribution (%) in *Pantoea* spp. Isolates**

| Isolate # | ***P.calida*** **containing the gene** | **Isolate #** | ***P*.agglomerans containing the gene** | ***Pantoea*** **spp. containing the gene** | **gene** |
|---|---|---|---|---|---|
| 1,4,5,6,7,9,14,15,17 | (%37.5)9 | 2,3,8,12,13,22,23,24 | (%33.33) 8 | (%70.83)17 | *hrc* |
| 4,6,7,9,14,15,17 | (%29.16) 7 | 10,11,12,19,22,24 | (%25) 6 | (%68.42)13 | *rcsA* |
| - | (0%) 0 | - | (0%) 0 | (0%) 0 | *rcsB* |
| - | (0%) 0 | - | (0%) 0 | (0%) 0 | *hpaA* |


**Figure2: Agarose gel electrophoresis of PCR assay show results of the *hrc* gene in the *Pantoea* spp. whereas M: Marker ladder 2000-100bp and drilling 1,2,3,4,5, 6, 7,8,9, 12,13,14,15,17, 22,23,24), isolates isolates Germ of *Pantoea* spp. Positive for the gene with a length of 647bp. Using an 80-ampere electric current and 100**

**voltage difference for one hour.**

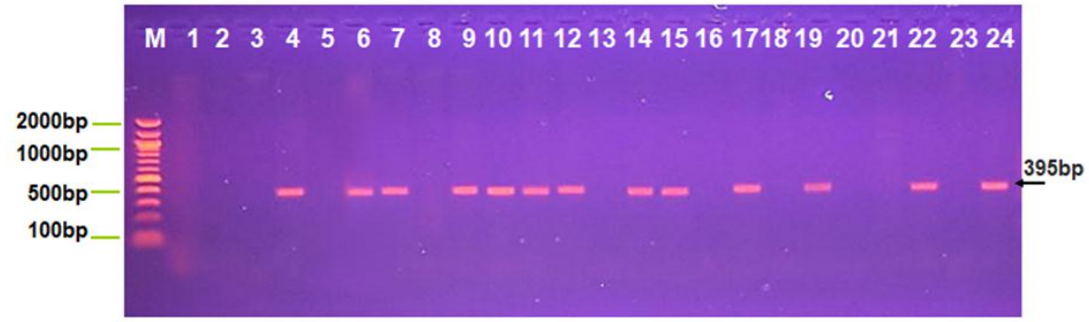


**Figure (3): Agarose gel electrophoresis of PCR assay show results of the *rcsA* gene in the . *Pantoea* spp. whereas M:Marker ladder 2000-100bp and drilling number 4, 6,7,9,10,11,12,14,15,17,19,22,24) isolates of *Pantoea* spp. germs for positive the gene with a length of 395bp. Using an 80-ampere electric current and 100 voltage difference for one hour.**


**Figure4: Agarose gel electrophoresis of PCR assay show results of the *rcsB* gene in *Pantoea* spp. whereas M: Marker ladder 2000-100bp and drilling number (1-24) are the isolates of *Pantoea* spp. germ is negative for the gene with a length of 438bp. Using an 80-ampere electric current and 100 voltage difference for one hour.**

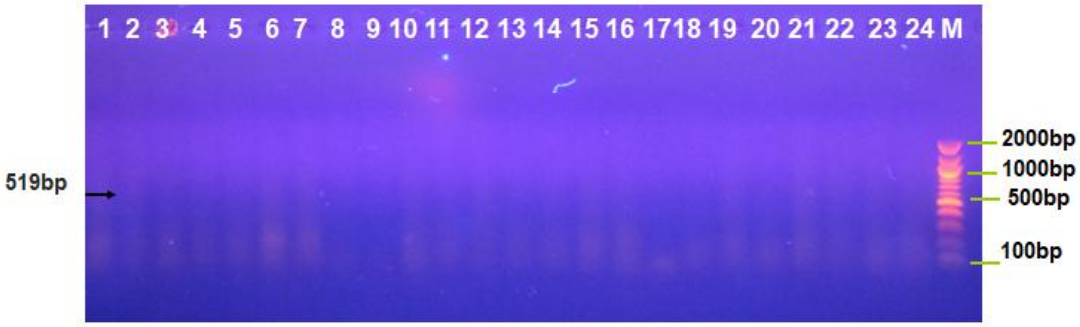

**Figure5; Agarose gel electrophoresis of PCR assay show results of the *hpaA* gene in *Pantoea* spp. whereas M: Marker ladder 2000-100bp and drilling number (1-24) are the isolates of *Pantoea* spp germ . is negative for the gene with a length of 519bp. Using an 80-ampere electric current and 100 volt difference for one hour.**

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