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 as 483 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. 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 swabs 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 (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 16S rRNA 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: CCGTGAACAAAGACTGAGGCT | 523bp | FR832419.1 |
|         | R: CGCTTCCTCTTTGATGCGCC | | |
|         | R: TTCCATGATGCGCCCTCATAT | | |
| rcsA    | F: AGATCCACCCGGTGGACGCTTT | 395bp | M60621.1 |
|         | R: CAATTTACGATGCTGCCG | | |
| rcsB    | F: GAACACACACCGCCGCATTTC | 438bp | Y09848.1 |
|         | R: CTTTGTATAAGGCCGCACGC | | U56662.2:3505-5535 |
| Hrc     | F: CGGGTGAATTTTATGCGCG | 647bp | |
|         | R: AGATGGGCTGTGCGTCATC | | |
| hpaA    | F: TGGCGAAGTAACGATGCGAT | 519bp | NC-003197.2:1195402-1196306 |
|         | R: AAAGTTTCAGTTCCACGCC | | |

F Forword, R: Reverse

**DNA Amplifying PCR Thermocycler Programs**

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

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

| Gene Name | Temperature (°C) / Time | Cycling conditions | | | | Cycles Numbr |
|-----------|------------------------|--------------------|-----------------|-----------------|-------------|
| 16srDNA   | Initial Denaturat-ion / time: 95/5 min | 95/30Sec. | 58/30Sec | 72/1min | 72 /5min | 35 |
| hrc       | Denaturation | Annealing | Extension | Final Extension / time | Extension | |
| 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 /min | 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 16S rRNA 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. Studies 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 | (% 37.59) | 2, 3, 8, 12, 13, 22, 23, 24 | (% 33.33) | 8 | (% 70.83) | hrc |
| 4, 6, 7, 9, 14, 15, 17 | (% 29.16) | 10, 11, 12, 19, 22, 24 | (% 25) | 6 | (% 68.42) | rcsA |
| - | (0%) | 0 | - | (0%) | 0 | rcsB |
| - | (0%) | 0 | - | (0%) | 0 | hpaA |

Figure 2: 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, 23, 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.
Figure 4: 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.

Figure 5: 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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