Determination some virulence genes (avrxacE2, avrxacE1, hrpG, acrAB) in Pantoea spp. isolated from hospitals of Al-Diwaniyah city, Iraq

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Abstract

Pantoea spp. is one of the opportunistic pathogens, which causes the spread of hospital infection due to its transformation from bacteria to plant organisms to human pathogenic bacteria. 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.s had been confirmed using API-20E Polymerase chain reaction technology. The current study reported the presence of two types of pathogens for the Pantoea spp.: the first is Pantoea agglomerans and Pantoea calida in hospitals in the city of Diwaniyah. Pantoea agglomerans was occurring in the studied isolates at a higher rate (2.27%) than Pantoea calida. All isolates of Pantoea spp were tested for the prevalence of virulence genes (avrxacE2, avrxacE1, hrpG, acrAB) using the polymerase chain reaction (PCR) technique. The highest incidence of hrpG was recorded as 79.16%, followed by avrxacE2 gene were 13 (54.16%). The current study did not record any presence of the virulence genes (avrxacE1 acrAB) among the isolates of Pantoea spp.

Keywords: Pantoea spp, avrxacE2, avrxacE1, hrpG, acrAB, Diwaniyah Iraq

Introduction

Pantoea spp. has been isolated from various environments and habitats including water, soil, dust, dairy products, meat, insects, animals and humans (1). they were bacteria that interacted with the plant and turned into pathogenic bacteria that could be the cause of human opportunistic infections due mainly to plant parts such as thorns or acquired infections in hospitals mostly from patients with poor immunity(2).

Pantoea spp. have genes that are responsible for pathogens, where hrp, and avr genes have been identified as genes related to pathogenesis and increased virulence of these bacteria inside the host. After injecting the proteins effectors encoded by these genes through the T3SS (Three type secretion system) in host cells, these active proteins inhibit the host's immune response, manipulating its cellular functions, altering host defenses to benefit pathogenic bacteria (3).
hrp genes are coded genes for the effectors proteins associated with infection of the third secretion system in T3SS. For the existence of these genes in the Pantoea bacteria, they transform from a plant-symbiotic bacteria previously called Erwina herbicola into pathogenic bacteria associated with pathogens of the hosts by the presence of the plasmid-borne pathogenesis island (PAI). These islands were isolated by increasing the virulence of the attacking bacteria of the host as a result of the presence of these functional genes, hrc genes and hrp genes that encode the T3SS proteins (4).

The avrxacE1 gene and avrxacE2 genes are synonymous with avr genes that encode a group of effective proteins associated with the pathogenesis of the Pantoea spp., as these proteins are encoded by these genes with resistance because the avr genes are changing the defense signal in the host resistor (5).

acrAB gene plays an important role in antibiotic resistance in Gram Stain-negative clinical isolates as well as environmental adaptation and increased virulence (6).

Isolation of Bacteria
Macaronkey agar and blood agar were vaccinated with sample swaps by projection method and incubated at 37°C for 18-24 hours. Dishes that did not show growth within 24 hours were incubated for another 24 hours before being taken as a negative result.

Bacterial Diagnosis
Bacterial colonies were identified according to shape, color, edges, textures and surface of the colonies, distinctive odors and growth or non-growth on differential media, selective media, blood decomposition on the bloodstream, lactose fermentation on the Maconkey medium. The microscopic characteristics of the cells, which included bacterial cell formation, regularity and arrangement of bacterial cells, and the nature of their interaction with Gram Stain (positive or negative) were observed. Biochemical tests including the Alcatelis test, Oxidase test, Hydrogen sulfide gas test, Mobility test, Carbohydrate fermentation test, Test set were used (7) (8).

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    | CCTGGGACAAAAAGACTGACGCT   | 523bp    | FR832419.1     |
| 16srRNA R    | CGCTTCTCTTTGTATGCGCC      |          |                |
Forward, 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 | Cycling conditions | Cycles Number |
|-----------|-------------------------|--------------------|---------------|
|           | Initial Denaturation / time | Denaturation | Annealing | Extension / Extension time | |
| 16s rRNA  | 95/5 min | 95/30Sec. | 58/30Sec | 72/1min | 72/5min | 35 |
| hrpG      | 95/5 min | 95/30Sec. | 58/30Sec | 72/1min | 72/5min | 35 |
| avrXacE1  | 95/5 min | 95/30Sec. | 58/30Sec | 72/1min | 72/5min | 35 |
| avrXacE2  | 95/5 min | 95/30Sec. | 58/30Sec | 72/1min | 72/5min | 35 |
| acrAB     | 95/5 min | 95/30Sec. | 58/30Sec | 72/1min | 72/5min | 35 |
Results and Discussions

Isolation and Identifications

Percentage of *Pantoea* spp. isolates was 3.85% (24 isolates), which were obtained from a total of 623 samples in Diwaniyah hospitals from different sources. These sources were 483 clinical samples and 140 environmental samples. Clinical samples included burns (11.23%), urine (13.16%) hemorrhagic fistula samples (13.32%), stool samples (4.33%), pus (4.97%), cough (11.87%), ear (7.86%), wounds (7.06%) and pharyngeal (3.69%). The environmental samples included medical tools (6.42%), beds (3.21%), walls (4.81%), medical equipment (3.21%) and floor (4.81%) as shown in Table (3).

The results of the current study showed that the *Pantoea* species bacterial is a dangerous opportunistic pathogen that may cause infection in cases of immunity weakening or when it reaches the sensitive areas of the body. The higher the risk of these infections is the high resistance to antibiotics used in treatment. The results also showed that the isolates of Gram Stain-negative bacteria were isolated (64.20%). Gram stain-positive bacteria were 132 isolates (21.18%), while the number of isolates without bacterial growth was 67 (10.75%). In general, information about *Pantoea* spp. is very limited in Iraq and this species is being taken as uncommon pathogenic factor that infects humans (9).

It was (10) who achieved 47.7% *Pantoea* spp. isolation from 40 isolates and recorded Gram Stain-positive bacteria (55.05%) from 289 isolates and non-growth bacteria (44.95%) from 236 isolates. He also isolated (11) samples of *Pantoea* spp. bacteria in the intensive care ward of Pneumonic.

Also, (12) reported the outbreak of *Pantoea* spp bacteria in hospitals, especially on dialysis patients.

Table 3: *Pantoea* spp. isolation (%) and positive, negative isolates on Gram Stain and non-growth isolates

| Source   | Total sample # / source | *Pantoea* spp. | Gram Stain negative | Gram Stain positive | Non-growth isolates |
|----------|-------------------------|----------------|---------------------|---------------------|---------------------|
| Burns    | (%11.23) 70             | (%)94.28 66    | (%)5.71 4           | -                   |
| Urine    | (%13.16) 82             | (%8.53) 7     | (%40.2) 33          | (%)24.39 20         | (%)26.82 22        |
| Fistula  | (%13.32) 83             | (%3.61) 3     | (%84.33) 70         | (%12.04) 10         | -                   |
| Stool    | (%4.33) 27              | (%7.40) 2     | (%77.77) 21         | (%14.81) 4          | -                   |
The current study aimed to investigate the prevalence and spread of virulence genes in 24 isolates of *Pantoea* spp. using the Monoplex polymerase chain reaction technique. Table 5 shows the distribution and spread of four genes (*avrxacE2, avrxacE1, hrpG, acrAB*) in the isolates of *Pantoea* spp. The number of isolates of these bacteria containing *avrxacE2* was 13 (54.16%). *P. agglomerans* was recorded 45.83% presence of this gene in 11 isolates. *P. calida* has recorded 8.33% presence in two isolates only (Figure 1). This result was similar to that indicated by (13) of the presence of *avrxacE2* gene in pathogenic bacteria, which contributes to the increase of virulence and the development of symptoms of the disease inside the host cells.

Also (14) pointed out to the expression of the amount of this gene as it is highly *P. agglomerans*. Studies have indicated that pathogenic bacteria express their disease in the host with virulence genes A that are encoded through A virulence proteins. These genes hinder the functioning of immune cells and destroy host cells, especially in the absence of resistance gene in the target host, which increases the ferocity and severity of the disease. Other studies have suggested that the expression of *avr* genes requires the presence of the Hrp pathway as its proteins are encoded through the T3SS (15).

*hrpG* was present in 19 isolates (79.16%), *P. agglomerans* was present in 12 isolates (50%) and *P. calida* was present in 7 isolates (29.16%) (Figure 2). This result was pointed out by (14) in that these bacteria contain this gene in high level in *P. agglomerans*. 

| Pus | (%4.97) 31 | (%6.45) 2 | (%70.96) 22 | (%22.58) 7 | - |
| Cough | (%11.87) 74 | (%2.70) 2 | (%67.56) 22 | (%29.72) 22 | - |
| Ear | (%7.86) 49 | - | (%59.18) 29 | (%24.48) 12 | (%16.32) 8 |
| Wounds | (%7.06) 44 | (%6.81) 3 | (%50) 22 | (%22.72) 10 | (%20.45) 9 |
| Pharyngeal | (%3.69) 23 | - | (%82.60) 19 | (%17.39) 4 | - |
| Medical tools | (%6.42) 40 | (%7.5) 3 | (%45) 18 | (%22.5) 9 | (%25) 10 |
| Beds | (%3.21) 20 | - | (%45) 9 | (%30) 6 | (%25) 5 |
| Walls | (%4.81) 30 | - | (%56.66) 17 | (%16.66) 5 | (%26.66) 8 |
| Equipment | (%3.21) 20 | (%10) 2 | (%55) 11 | (%35) 7 | - |
| Floor | (%4.81) 30 | - | (%43.33) 13 | (%40) 12 | (%16.66) 5 |
| Total | 623 | (%3.85) 24 | (%64.20) 400 | (%21.18) 132 | (%10.75) 67 |

**Virulence Genes in *Pantoea* spp. Isolates**
The *hrpG* gene regulates the work of the *hrp* gene pool in the host. Studies have shown that sequence analysis of a number of *hrp* genes for pathogenic bacteria of the host family showed a similarity of the sequences of *hrp* genes to human pathogenic bacteria as the regulatory genes *hrp/hrc* are encoded through the proteins of third enzyme secretory T3SS in pathogenic bacteria that are directly discharged into host cells, where regulatory genes are the key determinants of pathogenesis (16).

The current study did not record any presence of virulence genes (*avrxacE1,acrAB*) in *Pantoea* spp. isolates (Table 4). However, (13) indicated the presence of *avrxacE1* gene in isolated pathogens in their study.

The presence of the *acrAB* gene in pathogenic bacteria increases the opportunistic infections of the bacteria. Studies have indicated that the presence of this gene in pathogenic bacteria protects it from the host's toxic defenses and stimulates its slow growth in the presence of toxic compounds, antivirals, sterilizers or detergents. Bacteria indicates a mutant strain resistant to many toxins (17). Table 4 and figures (1), (2), (3), (4) respectively.

**Table 4: 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   |
|-----------|---------------------------------|-----------|-------------------------------------|-----------------------------------|--------|
| 7,9       | (%8.33) 2                       | 3,8,10,13,16,18,19,20,22,23,24 | (%45.83) 11                        | (%54.16) 13                      | avrxacE1 2 |
| 1,7,9,14,15,17,21 | (%29.16) 7                     | 2,3,8,10,11,12,13,16,18,19,20,22 | (%50) 12                          | (%79.16) 19                      | hrpG    |
| -         | (0%) 0                          | -         | -                                   | (0%) 0                           | avrxacE1 1 |
| -         | (0%) 0                          | -         | -                                   | (0%) 0                           | acrAB   |
Figure 1: Agarose gel electrophoresis of PCR assay show results for the avrXacE2 gene in the germ. *Pantoea* spp., whereas M: Marker ladder 2000-100bp and drilling number 3, 7, 8, 9, 10, 13, 16, 18, 19, 20, 22, 23, 24) germ isolates. *Pantoea* spp. positive for the gene with a length of 204bp and using an electric current 80 amp and 100 voltage difference for one hour.

Figure 2: Agarose gel electrophoresis of PCR assay show results of the *hrpG* gene in the *Pantoea* spp. whereas M: Marker ladder 2000-100bp and drilling number 1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22) bacterial isolates *Pantoea* spp. Positive for the gene with a length of 402bp. 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 *avrXacE1* gene in the *Pantoea* spp. germ, 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 304bp. 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 acrAB gene in the *Pantoea* spp. whereas M: Marker ladder 2000-100bp and drilling number (1-24) are the isolates of germ Pantoea spp. is negative for the gene with a length of 515bp. Using an 80-ampere electric current and 100 volt difference for one hour.

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