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Research Article

Detection of some virulence factors of *Pantoea* species isolated from urinary tract infections in the Iraqi population

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Abstract
Pantoea is an opportunistic bacterium primarily involved in nosocomial infections, impacting newborns and immunocompromised patients. The study aimed to investigate the virulence factor of *Pantoea* in urinary tract infections in hospitals in Al-Nasiriyah City, Iraq. The outcome appeared that 22 isolates out of 100 urine samples had been confirmed as *Pantoea* species by VITEK- 2. RcsA and hrpA genes were detected using conventional Polymerase Chain Reactions (PCR) and specific primers. The distribution of *rcsA* and *hrpA* was 40.9% and 59.1% respectively. The *Pantoea* species were distributed according to age, gender, and ABO blood groups. The percentage was 72.3% for ages 40–70, and females recorded 54.5% more than males. Individuals with the A blood group had a higher percentage (50%) than other blood groups; there were two types of variations (Transversion and Transition) of the16S rRNA gene. It was determined through the sequence alignment, and the symmetry was 90% with the Italian isolate of *Pantoea* species LL92 (ID: KF202812.1). These variations in the *Pantoea* local Iraqi isolate were registered in NCBI (ID: OM971851).This bacterial genus recently descended from the species of *Enterobacter* sp.. The isolation of *Pantoea* sp.was from UTIs, while previously, in most studies, they were isolated from the surrounding environment.

Keywords: *hrpA, Pantoea, rcsA,* Transition**,** Transversion, Urinary tract infection (UTI)

INTRODUCTION

Pantoea species are gram-negative bacteria, elective anaerobic, and rods. They belong to the *Enterobacteriaceae* family (Dutkiewicz *et al.,* 2016). Opportunistic bacteria are the most frequent cause of nosocomial infections in newborns and immunocompromised patients (Mani and Nair,2021). Infections in humans are rare, but these could be related to an injury from farming or children's games, as well as incidental bacteremia from medical tools like catheters and contaminated intravenous solutions (Dutkiewicz *et al*., 2016). *Pantoea* spp. are habitually found in the skin, urinary, respiratory, and gastrointestinal tracts of patients who are in hospitals. These locations are considered the entrance to invasive diseases (Cunningham and Leber,2018). In recent years, several epidemics were recorded as results of *Pantoea* spp. as the prevalence of healthcare-associated *Pantoea agglomerans* bloodstream infections in 12 malignant patients. Urinary tract infections (UTIs) are the first cause of morbidity in older men and females of all ages. Serious sequelae include multiple recurrences, renal damage in children, pyelonephritis with sepsis, Other factors such as gender, age, diabetes, Catheter-associated urinary tract infection (CAUTI), genitourinary tract (GUT) abnormalities and hospitalization status, a high risks or recurrent UTIs (Siwakoti *et al*.,2018). Known transcription factor *rcsA* regulates capsule (rcs) synthesis. It has a role in synthesizing colonic acid and (K) antigen of capsular polysaccharide (cps) in *Salmonella typhi* and *E.coli* (Kernell Burke *et al.*,2015). Also, *rcsA* has represented the main factor in the virulence of *Pantoeastewartia*. The virulent strain lacking exopolysaccharide (EPS) production has gene disordering in the *rcsA* or *cps* locus. The hrp genes stimulate HR (hypersensitivity response) in resistant host and nonhost plants (Li *et al.,* 2011). The hypersensitivity response (HR) is fast apoptosis that is stimulated depending on the discrimination of the pathogen. The hrp genes are very well preserved in animal and plant bacterial pathogens. These are recognized as hrp -conserved genes (hrc). These genes encode TTSS

(type III secretion system) and have a major role in pathogenicity and disease stimulation(Pesce *et al.,* 2017).

Pantoea spp. has genes responsible for pathogenesis, such as hrp and rcs genes, identified as genes associated with pathogenesis and increased virulence of these bacteria inside the host. After injection of effectors of proteins encoded by some of these genes through the T3SS , (Three type secretion system) in host cells, these active proteins inhibit the host's immune response, manipulate their cellular functions, and alter host defenses to benefit, pathogenic bacteria (Schwarz *et al*.,2010) . The present study aimed to investigate the virulence factor of *Pantoea* spp. through molecular technique in UTIs of patients in hospitals of Al-Nasiriyahcityin, Iraq.

MATERIALS AND METHODS

Collection of samples

From April to December 2020, 100 urine specimens were collected from patients with urinary infections(UI) who visited hospitals in Al-Nasiriyah City, Iraq. Information was recorded, including age, sex, ABO blood group. Urine samples were put in a sterile urine container and transferred as fast as possible to the laboratory to complete diagnosis.

Ethical approval

Ethical approval was obtained from the Scientific Research Ethics Committee of the Iraqi Ministry of Health by 2421 in February 2020.

Isolation and identification of bacteria

Bacteria were isolated and identified according to microscopic and cultural characteristics by growing on differential media such as MacConkey medium. Biochemical tests includingIMVC., Oxidase, catalase, motility and carbohydrate fermentation. Diagnosticswereconfirmed by the VITEK-2 system(Brown , 2007;MacFaddin , 2000).

Molecular detection

The DNA was extracted from samples using MiniPrep Quick-DNA (Cat. No. D3024; Zymo, USA). *Pantoea* genes were detected by PCR thermocycler amplification (Table1), and the conditions of PCR reaction for genes are shown in Table 2. The reaction contents for each gene involved Taq PCR PreMix,(2X) 5µl from iNtRON, Korea, forward primer 1 µl (10 picomols/µl), reverse primer 1 µl,(10 picomols/µl), DNA sample was 1.5µl. Finally, the free nuclease water was 16.5 µl, and the final reaction volume was 25µl. The agarose gel (2%) was used for electrophoresis to visualize the PCR products when stained via Red Safe (Intron, Korea). The sequence of the products of PCR was performed by Macrogen DNA sequencing (Korea). The nucleotide sequence was aligned using the NCBI's ,Basic Local Alignment Search Tool (BLAST) program to identify the sample and submit it to GenBank (ID). The associated sequences of the sample were obtained from NCBI (www.ncbi.nlm.gov/nucleotide) and included in the multiple alignments using MEGA6 program (Tamura *et al*., 2011).

RESULTS AND DISCUSSION

Diagnosis of *Pantoea* sp.

The results showed that 22 out of 100 samples were diagnosed as *Pantoea* species using VITEK-2 System with a probability of 86%. Also, it was diagnosed via PCR using 16srRNA.The findings are illustrated in Fig.1. These isolates had 16srRNA at a molecular weight of 1250 bp. Manyother studies demonstrate the VITEK-2 technique and VITEK-2 ID cards, which can supply credible and exact outcomes for gram-positive cocci and gram-negative bacilli (Ling *et al.,* 2001). Also, it was detected as *Pantoea* sp. by 16s rRNA. Delétoile *et al*. (2009) used multi-locus gene sequencing and 16 srRNA to diagnose *Pantoea* in their study. The conventional techniques have represented the complex methods in identifying *Pantoea* with accuracy. VITEK-2 and the 16S .rRNA gene sequences were the best ways to

Table 1. Sequences primers of the study genes

Gene name	Sequence	Tm (°C)	GC (%)	Product size	References
16srRNA	F5'- AGAGTTTGATCCTGGCTCAG- 3' R5'- GGTTACCTTGTTACGACTT- 3'	54.3 49.4	50.0 42.1	1250bp	(Srinivasan et *al.,*2015)
rcsA	F5'- AAGTCCATCCGTTGACGCTT - 3' R 5'- CAATTTACCGATGGCTGCCG- 3'	55 50	50 49	395bp	(Al-baaj and Al-Ramahy, 2019)
HrpA	F5'- TGGGCAGTAACGATGTGCAT - 3' R5'- AAAGTTTCAGTTCACCGCGC - 3'	55 51	52 45	519bp	(Al-baaj and Al-Ramahy, 2019)

Table 2. Conditions of PCR reaction for the studied genes

Genes names	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
16srRNA	94°C/3 min 1cycle	94°C/45sec 35cycle	58°C/45sec 35cycle	72 °C / 1min 35 cycle	72°C/7min 1 cycle
rcsA hrpA	94°C/5 min 1 cycle	94°C/30sec 35cycle	58°C/30sec 35cycle	72 °C / 1min 35cycle	72°C/7min 1cycle

identify *Pantoea* dispersa (Yang *et al.*,2022).

Virulence detection and distribution of *Pantoea* sp.

The outcomes indicated approximately twenty-two isolates with virulence genes *rcsA and hrpA*, as shown in Fig.2 and 3. The distribution of *rcsA* and *hrpA* was 40.9% (9/22) and 59.1% (13/22), respectively.

Regarding genetic variation detection results, the sequence alignment revealed two variations (transversion and transition) in the 16S rRNA gene. Moreover, the symmetry was 90% with the Italian isolate *Pantoea* sp. LL92 (ID: KF202812.1) in GenBank. The locations of the various types are shown in Table 3 and Fig.4. Furthermore, these differences in *Pantoea* local Iraqi isolates were registered in NCBI under the ID: OM971851 (https://www.ncbi.nlm.nih.gov/nuccore/OM971851.1/).

Concerning the outcomes of the distribution of *Pantoea* sp. according to age, gender, and ABO blood groups, it was observed that the ages ranged from 40-70 years, and the percentage was 72.3% highly than the ages 25-39 years that, was the percentage 27.3%. In addition, females were recorded at 54.5% more than males at 45.5%. Besides, the individuals in the A blood group were 50% more than the B group, 13.6%, and the O group, 36.6% (Table 4).

The study showed that the *Pantoea* species bacterial is an opportunistic pathogen that may cause infection in weakened immunity or when interacting with the body's sensitive areas. The use of urinary catheters was the most frequent source of infections and Gv- bacteria was the most common cause. UTIs are a function of the duration of catheterization.Long-term catheterization may lead to a failure of natural defense mechanisms and be a site of survival for bacteria by biofilm formation (Bonkat *et al*., 2018).

Colony morphology showed that *Pantoea*spp. appeared as convex mucoid, pink colonies on MacConkey agar because the bacteria were lactose fermenters. In biochemical tests, the negative results for indole production are due to the bacteria's inability to produce tryptophanase, which deaminates tryptophan to produce indole. The methyl red (MR +) test was positive due to the production of the complete acid during the fermentation of glucose. Voges-Proskauer (VP+) was positive due to the bacteria's ability to produce acetone. Buyukcam *et al*.(2018) explained that *P. agglomeransis* is an important cause of UTIs in Turkish hospitals. Generally, information about *Pantoea* spp. is very limited in Iraq, and this species is being taken as an uncommon pathogenic factor that infects humans.

RcsA is the positive effector of EPS (extracellular polysaccharide) composition in the *Enterobacteriaceae*. The protein RCS A has three conserved domains. It ends with an open read framing C-terminus that shares a high amino acid homologous to the DNA helix-binding motif of bacteria stimulator proteins. The

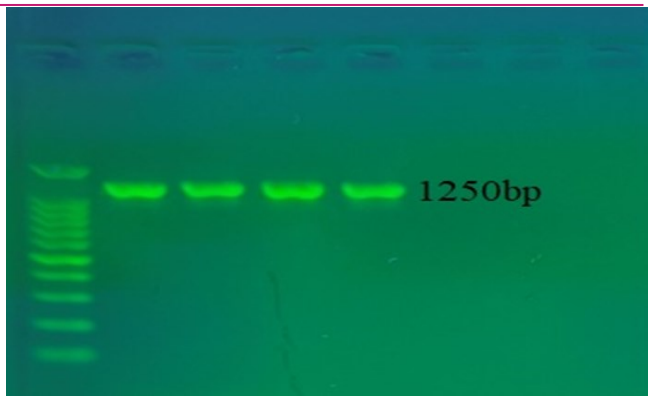

Fig. 1. *Product of PCR (1250 bp)for some specimens from 22 samples containing 16srRNA;Electrophoresis on 2% agarose in 1x TBE buffer at 5 volt/cm2 for 1:30 hours; DNA ladder (100plus)*

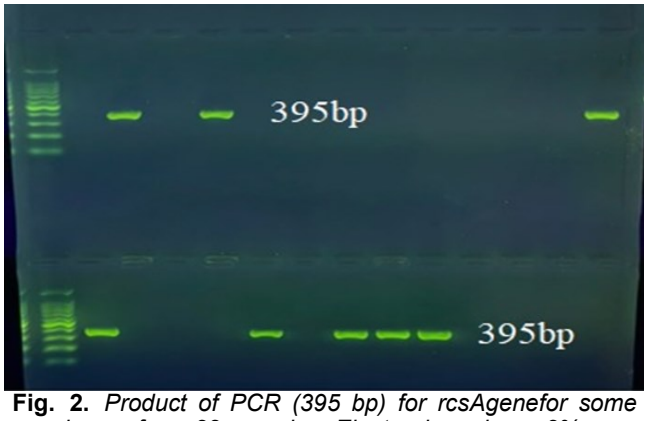

Fig. 2. *Product of PCR (395 bp) for rcsAgenefor some specimens from 22 samples; Electrophoresis on 2% agarose in 1x TBE buffer at 5 volt/cm2 for 1:30 hours; DNA ladder (100plus)*

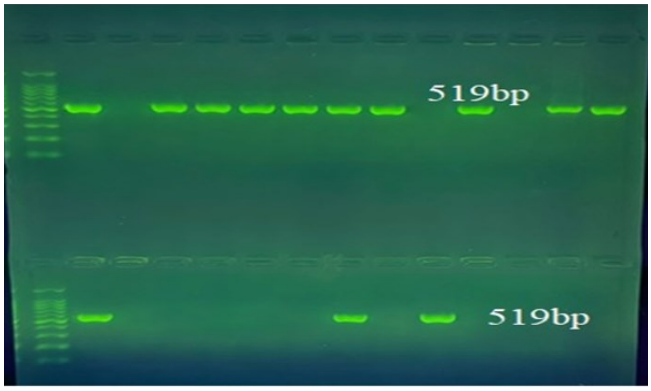

Fig. 3. *Products of PCR (519 bp) for hrpA gene for some specimens from 22 samples; Electrophoresis on 2% agarose in 1x TBE buffer at 5 volt/cm2 for 1:30 hour; DNA ladder (100plus)*

inactivation of RCS A via insertion mutagenesis reduced EPS production and eliminated *Pantoea* sp. virulence. EPS production is considered a general feature of gram-negative bacteria (Kim *et al*., 2017), which generates a suitable microenvironment for bacteria and their protection from dehydration, outer stress, and mechanisms of defense in the host (Nguyen *et al*.,2020). Therefore, EPS is the primary determinant for the virulence of bacteria in many interactions between

Table 3. Alignment analysis and location type variations of *Pantoea* sp 16SrRNA gene

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
4	Transversion	322	T/G	ID: KF202812.1	Pantoeasp. LL92 16SrRNA gene	90%
	Transversion	339	G/T			
	Transversion	341	C/G			
	Transversion	345	A/C			
	Transversion	346	A/G			
	Transversion	352	G/T			
	Transversion	359	A/C			
	Transition	362	T/C			
	Transversion	364	G/C			
	Transition	370	G/A			
	Transition	372	G/A			
	Transition	384	T/C			
	Transversion	386	G/C			
	Transversion	396	A/T			
	Transition	402	T/C			
	Transversion	407	G/C			
	Transition	414	A/G			
	Transversion	417	G/C			
	Transversion	419	G/C			
	Transition	421	A/G			
	Transition	433	T/C			
	Transversion	437	G/C			
	Transversion	438	G/T			
	Transversion	448	A/T			
	Transversion	452	G/C			
	Transition	454	G/A			
	Transversion	455	G/C			

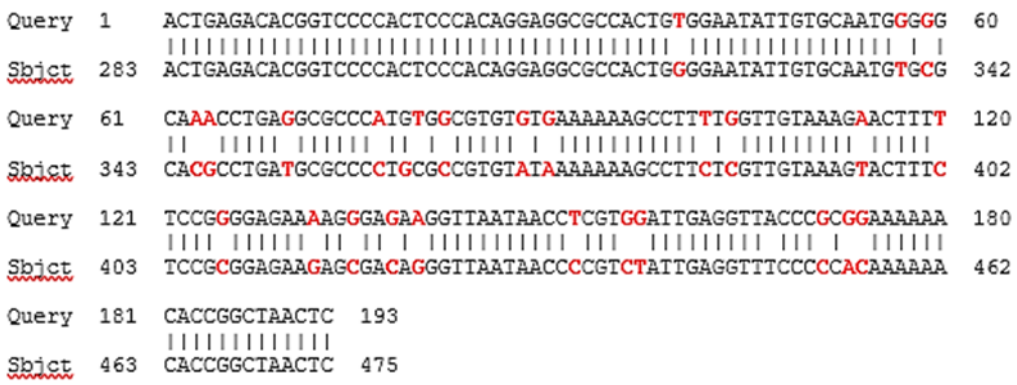

Fig. 4. *Alignment analysis and location variation of Pantoea sp. 16SrRNA gene*

host and pathogen, like *Erwinia amylovora* and *Pantoea stewartia* (Kim *et al.,* 2017). The Rcs (Regulator capsule synthesis), a non-classical two-constituent regulation system (TCS) established in *Enterobacteriaceae*, is considered one of the envelope stress response pathways. Rcs systems detect envelope disruptions and organize the transcriptome to prevent stress, which is considered especially important for survival. and pathogenic bacterial virulence (Meng *et al*., 2021).

The gene hrp (hypersensitivity and pathogenicity) encodes constituents of the system secretion type III, which are named *hrp, hrc* (hrp-conserved), or hpa (hrp-associated). System secretion Type III is a significant virulence factor for bacteria (gram-negative) infecting their host (Piqué *et al*., 2015). Kang *et al*. (2020) observed this in their study. The effect of anti-virulence factors on the expression of the *hrp A* gene and other genes related to the type III, secretion system inhibited their transcription and led to a decline in virulence. Moreover, *Pantoea* can enter human and animal organs as an opportunistic pathogen, which causes dan-

Table 4. Distribution of *Pantoea* according demography data

Variables	Total number=22
Ages (25-39)	6(27.3%)
(40-70)	16(72.3%)
Gender	
Female	12(54.5%)
Male	10(45.5%)
ABO groups	
A+	11(50%)
B+	3(13.6%)
O+	8(36.6%)

gerous and sometimes deadly infections. The most acute infections occur in young people and individuals with other diseases. It can be isolated from a wide diversity of ecological places and several types of specimens for humans and animals (Mardaneh and Dallal, 2013).

In *Pantoea*sp. also, there were two variations (Transversion and Transition) of the 16S rRNA gene. It was determined through sequence alignment. Moreover, the symmetry was 90% with the Italian isolate of *Pantoea* sp. LL92 (ID: KF202812.1). Furthermore, these differences in *Pantoea* local Iraqi isolates were registered in NCBI as ID: OM971851.

Studies(Rakhashiya*et al*., 2016; AthraaAbdulamir *et al*., 2020) showed that sequence analysis of many *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 (Barash and Manulis-Sasson, 2007; Moreno-Pérez *et al.,* 2021)

In some studies, *Pantoea agglomerans* infections were diagnosed as common infections in wound inflammation at 35.7%, urinary infections at 21.4%, and pneumonia at 21.4% (Buyukcam *et al., 2018).* Cheng *et al.* (2013) observed an increase of A blood group persons at 44.4% compared with other blood groups. Another study indicated that the infection of *Pantoea agglomerans* was 7–88 years old (Mirtella *et al.,* 2021). The study shows that females are more likely to develop a UTI than males and agree with the findings of Foxman, 2014) .Those who reported outbreaks of UTI were more present in females.

The space between the anus and the urethral meatus in females is short and long in males; the wet content peripheral of the urethra; and genetic tendency (as in the ABO blood group) are all factors related to the risk of urinary infections (Ghasemi *et al*., 2009). Benli *et al.* (2019) noticed the association between lower urinary tract infection and blood group A. Another study indicated that O blood groups in pregnant women were more frequent than B blood groups with urinary infections (Mahmoudian *et al.,* 2021).

The present study is important and different from other research because *Pantoea* spp. is limited in Iraq, and this species is being taken as a non-recurring pathogenic factor that infects humans.Therefore, virulence genes rcsA and hrpA, which are more dangerous in their effects on humans, were selected, and there are few studies on these genes of *P.agglomeransis,* an important cause of UTIs in some hospitals in other countries, but information about them is very limited inside Iraq. Hence, the study focused on some virulence genes.

rcsA gene responsible for regulating capsule synthesis, 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 and the hrp gene encodes components of the type III secretion system, called hrp, which is an important virulence factor for Gram-negative bacteria that infect their host.The present study choice is *Pantoea* specifically; previously, it infected plants. Most recent studies have shown that *Pantoea* has virulence genes important in causing human disease.

Conclusion

The present study demonstrated the possibility of isolating *Pantoea* sp. from UTIs patients and identified it using VITEK-2 system and *16SrRNA* gene sequences. The conventional PCR technique observed the moderate presence of virulence genes (rcsA and hrpA).Genetic variation was detected, showing two different types (transversion and transition) of the 16S rRNA gene. The sequence alignment indicated the homology was 90% with the Italian isolate *Pantoea* sp. The prevalence of Pantoea sp. was in the age group (40-70), females and A blood group. The Pantoea genus recently descended from *Enterobacter* because it had most of its diagnostic characteristics. *Pantoea* isolates from urinary tract patients have virulence genes important in causing the disease, although they were previously isolated from plants and the environment.

Conflict of interest

The authors declare that they have no conflict of interest.

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