

Isolation of MDR *Providencia stuartii* from tracheal aspirates of two hospitalized patients

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ABSTRACT

The emergence of nosocomial infection due to Multi-Drug Resistant (MDR) bacteria is a challenge to infection control. This study presented two *Providencia* species from two hospitalized patients admitted to Imam Hussein hospital in Iran. Biochemical and molecular tests were performed for the identification of the strains. The resistance pattern of these isolates was determined using an antimicrobial susceptibility test. Both *P. stuartii* isolates were resistant to all antibiotics. Among carbapenemase resistance genes, *bla_{NDM}*, *bla_{GES}* and *bla_{KPC}* were detected in both of them. One isolate was able to transfer the resistant gene in the conjugation test. The presence of MDR strains among nosocomial infection agents such as *P. stuartii* isolates could affect the health care system efficiency.

Keywords: *Providencia stuartii*; MDR; carbapenemase resistance genes

INTRODUCTION

Providencia species are Gram-negative bacteria that belong to the

Enterobacteriaceae family. This genus is a rare nosocomial infection agent, although isolation of these bacteria among hospitalized patients is critical because it causes different infections in multiple

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anatomic sites [1-3] such as bacteremia, skin, and Urinary Tract Infection (UTIs), wound and pneumonia. Patients with severe underlying diseases are susceptible to various nosocomial infections. Invasive procedures including dialysis and catheter can be risk factors for the development of this kind of infection [4-6]. The emergence of antimicrobial resistance leads to limitations in the effectiveness of antimicrobial agents; therefore, the wide spread of antibiotic resistance could become a significant public health threat [7]. The emergence of MDR *Providencia* species harboring Carbapenem resistance genes in hospitalized patients causes various difficulties in the treatment of related infections. This study aimed to investigate the presence of pathogens and their antimicrobial features in two hospitalized patients with underlying diseases.

Case presentation

Two patients, a male and a female were admitted to Imam Hussein hospital with the underlying diseases in 16 and 31 October, respectively. The female patient was 74 years old with a history of diabetes and pneumonia. The male patient was 51 years old which had lymphoma, weakness, and lethargy symptoms. Both patients were

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hospitalized in ICU. Tracheal aspirates and blood samples were collected and sent to laboratory for bacterial isolation and both two patients underwent CT examination. Also, in order to choose appropriate *antimicrobial* agents for suspected infection, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, antimicrobial susceptibility testing was performed using the disc diffusion method. Antibiotic discs including levofloxacin (10 mg), imipenem (500 mg), cotrimoxazole (960 mg), piperacillin-tazobactam (3.375 g), cefotaxim (2 mg), ampicilin (2 mg), amoxicillin (50 mg) and ceftazidime (150 mg). *Escherichia coli* ATCC 25922 was used as the reference strain for antimicrobial susceptibility testing. After that, PCR molecular assay was performed for isolates confirmation and identification.

Laboratory Findings

Two *P. stuartii* strains were isolated from *tracheal* samples of both patients. Conventional biochemical and molecular tests confirmed isolates as *P. stuartii*. Antimicrobial susceptibility test was performed against levofloxacin (10 mg), imipenem (500 mg), cotrimoxazole (960 mg), piperacillin-tazobactam (3.375 g), cefotaxim (2 mg), ampicilin (2 mg),

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amoxicillin (50 mg) and ceftazidime (150 mg). The isolated strains were resistant to all tested antimicrobial agents. In other words, *P. stuartii* isolates in both patients were multidrug-resistant. No pathogen was detected in blood culture. The Carbapenemase genes were detected using specific PCR primers (Table 1). PCR products were sequenced and the obtained results from this study were compared to gene bank sequences.

In this study, *bla*_{GES}, *bla*_{KPC} and *bla*_{NDM} genes were detected in both isolates. Other

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carbapenemase resistance genes including *bla*_{VIM}, *bla*_{IMP}, *bla*_{CarO}, *bla*_{DacD} and *bla*_{OXA} were not detected. Conjugation test was performed to investigate the bacterial ability in gene transfer. *P. rettgeri* isolated from the first patient had conjugative plasmids. Despite several antibiotic usages and other critical care assistance in ICU, both patients died due to decreased level of consciousness.

Table 1. Primer sequence, length, and annealing temperature used in this study

Primer name	Sequence (5' →3')	DNA amplicon size (bp)	Annealing temperature (°C)	Reference
Oxa-143-like	F: TGGCACTTTCAGCAGTTCCT R: TAATCTTGAGGGGGCCAACC	150	52.2	[8]
OXA-23-like	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCAT	501	53.2	[8]
OXA-24-like	F: GGTTAGTTGGCCCCCTTAAA R: AGTTGAGCGAAAAGGGGATT	249	51	[8]
OXA-58-like	F: AAGTATTGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599	56	[8]
NDM	F: CGGAATGGCTCATCACGATC R:CGGAATGGCTCATCACGATC	621	50	[8]
KPC	F: CGTCTAGTTCTGCTGTCTTG R:CTTGTCATCCTTGTTAGGCG	798	55	[8]
VIM	F: GATGGTGTGTTGGTCGCATA R:CGAATGCCGAGCACCAG	390	55	[8]
IMP	F: GGAATAGAGTGGCTTAAYTCTC R:GGTTTAAAYAAAACAACCACC	232	55	[8]
GES	ATGCGCTTCATTC CACGCAC CTATTGTCCGTGCTCAGG	846	55	[8]
OXA-51- like	F: CTAATAATTGATCTACTCAAGTTAC R: GAATACTCCATTTGAACCARTGG	988	55.1	[8]
CarO	F: GACAACACTACAGCTTTACTTGCT R: ACACCAACTTTACCAACTGG	711	57	[9]
DacD	F: ACTACTCTTACCAATGCTGCTCTAC R: TGAGAATAGGCTTGAGAACCACATC	1218	59	[9]

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This is the first human case report of *Providencia* infection in Iran. In this study we obtained two *Providencia* isolates from a tracheal sample of two hospitalized patients in ICU with underlying disease. Two isolates of this study were MDR and among MDR isolates, Carbapenem resistance genes (bla_{NDM} , bla_{GES} and bla_{KPC}) is affirmed in this study. Carbapenem resistance in *Enterobacteriaceae* is major health challenge [10]. The first case of Carbapenem resistant in *Providencia* was detected in Japan 2003 [11]. Moreover, in the study conducted by Tshisevha in 2017 [7], four *Providencia* isolates were detected in four hospitalized patients. All strains were MDR and positive for carbapenemase genes.

Studies showed the prolonged hospitalization ranging from 24 to 106 days, can affect the acquisition of Carbapenem resistant *P. stuartii* [6,12]. Common equipment such as catheter and dialysis machine can facilitate the spread of Carbapenem-resistant *Providencia*. Several studies showed that in patients with urinary catheters, Carbapenem-resistant *Providencia* is detected [13].

Shin *et al* in 2018 reported that eight *P. rettregi* isolates showed high resistance to

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multiple antibiotics which harbored bla_{NDM} and bla_{per} [14]. Meanwhile, bla_{KPC} , bla_{NDM} and bla_{GES} genes were detected in both isolates. The importance of carbapenemase genes is due to potential transferability to other species by mobile genetic elements like plasmids and transposons. This process named Horizontal Gene Transfer (HGT), which has remained key agent of bacterial virulence genes and genetic properties acquisition and bacterial evolution [15]. Mentioned reports confirm the presence of transposon elements harboring resistance factors which can distribute among patients and health workers and can spread in hospitals environment and transfer to society. Also, in the present study, one isolate was able to transfer resistance genes. Treatment of Carbapenem-resistant *Providencia* depends on bacterial susceptibility against antibiotics. Meropenem is an effective procedure for carbapenem-resistant treatment [15]. Several studies showed that combination therapy have good outcomes in patients. The study conducted by Zavascki *et al*, showed that among five patient, three of them were treated with combination of piperacillin/tazobactam and Meropenem. One patient received combination of amikacin and imipenem and other one

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received levofloxacin and only first three patients had good results [3]. In addition, Douka *et al* reported that most effective combination was piperacillin/tazobactam with amikacin, which eradicate *Providencia* isolates from all infected patients [13]. The choice of the best antibiotic for the treatment of bacterial infections relies on the antibiogram results. To reduce prevalence of resistance genes, reduces use of antibiotics and control of drug consumption is necessary [16-18].

CONCLUSION

A serious public health threat, is the emergence of antimicrobial resistance in pathogenic bacteria. Nosocomial infection due to MDR *Providencia* is a challenge to patient treatment and infection control.

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