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, blaNDM, blaGES and blaKPC 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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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), 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.
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, *blaGES*, *blaKPC* and *blanDM* genes were detected in both isolates. Other carbapenemase resistance genes including *blavIM*, *blaIMP*, *blaCarO*, *blaDacD* and *blaOXA* 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

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5' →3')</th>
<th>DNA amplicon size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxa-143-like</td>
<td>F: TGGCACTTTTCAGCAGTGTCCT R: TAATCTTGAGGGGCGCAACC</td>
<td>150</td>
<td>52.2</td>
<td>[8]</td>
</tr>
<tr>
<td>OXA-23-like</td>
<td>F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTGCT</td>
<td>501</td>
<td>53.2</td>
<td>[8]</td>
</tr>
<tr>
<td>OXA-24-like</td>
<td>F: GGGTTAGTGGCCCCCTTAAA R: AGTGGAGCGAAAGGGGATT</td>
<td>249</td>
<td>51</td>
<td>[8]</td>
</tr>
<tr>
<td>NDM</td>
<td>F: CGGAATGGCTCATCAACGATC R: CGGAATGGCTCATACAGATC</td>
<td>621</td>
<td>50</td>
<td>[8]</td>
</tr>
<tr>
<td>KPC</td>
<td>F: CGTCTAGTTCTGCTGTCTTG R: CTTGTCATCCTTGTTAGGCG</td>
<td>798</td>
<td>55</td>
<td>[8]</td>
</tr>
<tr>
<td>VIM</td>
<td>F: GATGGGTGTTGGCTGCGATA R: GGAATGCACCGCAACGACG</td>
<td>390</td>
<td>55</td>
<td>[8]</td>
</tr>
<tr>
<td>IMP</td>
<td>F: GGAATGAGTGGGCTTAAAYTCCT R: GGTITAAAYAAAACACACC</td>
<td>232</td>
<td>55</td>
<td>[8]</td>
</tr>
<tr>
<td>GES</td>
<td>ATGGCCTTCAATCCAGCGAC CTATTGTCCTGCTGTCAGG</td>
<td>846</td>
<td>55</td>
<td>[8]</td>
</tr>
<tr>
<td>OXA-51-like</td>
<td>F: CTAATATTGATCTACTAAGTTAC R: GAATACTCCATTGGACARTGG</td>
<td>988</td>
<td>55.1</td>
<td>[8]</td>
</tr>
<tr>
<td>CarO</td>
<td>F: GCAACTACAGCTTACCTTGCT R: ACACCAACTTCCAACTTTG</td>
<td>711</td>
<td>57</td>
<td>[9]</td>
</tr>
<tr>
<td>DacD</td>
<td>F: ACTACTCTTTACCATCTGCCTCTAC R: TGGAATAGGGTGGAGAACCACATC</td>
<td>1218</td>
<td>59</td>
<td>[9]</td>
</tr>
</tbody>
</table>
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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 (blaNDM, blaGES and blaKPC) 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 multiple antibiotics which harbored blaNDM and blaPER [14]. Meanwhile, blaKPC, blaNDM and blaGES 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 susceptibly 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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Rohani et al. 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 nessesary [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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