Laboratory investigation of a suspected outbreak caused by Providencia stuartii with intermediate resistance to imipenem at a long-term care facility

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KEYWORDS
- carbapenem resistance
- long-term care facility
- multidrug-resistant organisms
- outbreak
- Providencia stuartii

Abstract  
Background: Providencia stuartii survives well in natural environment and often causes opportunistic infection in residents of long-term care facilities (LTCFs). Clinical isolates of P. stuartii are usually resistant to multiple antibiotics. The bacterium is also naturally resistant to colistin and tigecycline. Treatment of infections caused by carbapenem-resistant P. stuartii is challenging.

Methods: During a 15-month period in 2013–2014, four isolates (P1, P2, and P3B/P3U) of P. stuartii showing intermediate resistance to imipenem were identified at a regional hospital in southern Taiwan. They were identified from three patients (P1–P3) transferred from the same LTCF for the treatment of the infection. Pulsed-field gel electrophoresis was used to genotype the isolates. Resistance genes/plasmids and outer membrane proteins were investigated by polymerase chain reaction and sequence analysis.

Results: Isolates P1 and P3B/P3U demonstrated similar pulsotypes. All isolates were found to have resistance genes ($\text{bla}_{\text{CMY-2}}$, $\text{qnrD1}$, $\text{aac(6')-lb-cr}$) carried on nonconjugative IncA/C.
Introduction

With the extension of human life, growing demand for long-term care facilities (LTCFs) appears inevitable. Residents of LTCFs are usually with advanced ages and are associated with a variety of clinical disabilities, chronic diseases, and compromised immune status, making them vulnerable to a variety of infections. The generally close living proximity, frequent contact between nursing staff and residents, and less optimized infection control measures may facilitate the spread of microbial organisms and increase infection burden. These conditions may lead to the necessity for frequent prescription of antimicrobial agents. However, diagnosis of infection among these elderly residents is often difficult, resulting in the increase of inappropriate antimicrobial use and the development of multidrug-resistant organisms (MDROs). The frequent visits or transfers between LTCFs and acute-care settings may further worsen the problem in terms of infection acquisition or resistance development.

Providencia stuartii, a Gram-negative bacterium belonging to the family of Enterobacteriaceae, can survive well in natural environment, including water and soil. The bacterium often causes opportunistic infection in hospitalized patients or LTCF residents. Particularly among those with indwelling urinary catheters, P. stuartii could cause bacteriuria and further develop into bacteremia. Any flaw in the infection control measures may result in cross infection among the patients or even lead to infection outbreaks.

Clinical isolates of P. stuartii are often resistant to multiple antibiotics. Despite the natural presence of chromosomal AmpC genes, P. stuartii could also acquire various exogenous resistance genes and develop the resistance to multiple antimicrobial agents, including carabapenems. Clinically, colistin and tigecycline are usually considered for infections caused by carbapenem-resistant Enterobacteriaceae (CRE). However, P. stuartii also possesses natural resistance to these two antibiotics. In other words, when treating infections caused by carbapenem-resistant P. stuartii, clinicians may fall in a dilemma that no appropriate antibiotic is available.

At a regional hospital in southern Taiwan, two isolates of P. stuartii that showed intermediate resistance to imipenem were identified from blood specimens of two patients in May and June 2014, respectively. The two patients were referred from the same LTCF for the treatment of the infection. As the situation was relatively rare, the occurrence of an outbreak in the LTCF was highly suspected. Retrospective analysis revealed that in March 2013, another imipenem-intermediately resistant P. stuartii isolate had been identified from another patient also from the LTCF. The present study was therefore performed aiming to elucidate the relationship of these isolates and the underlying resistance mechanisms.

Materials and methods

Bacterial isolates and patients

Four isolates of P. stuartii showing intermediate resistance to imipenem were studied. The first one was identified in March 2013 from the abscess specimen of the first patient (P1). The second one was isolated from the blood specimen of another patient (P2) in May 2014. In June 2014, two more isolates were identified from the blood (P3B) and urine (P3U) specimens of a third patient (P3). The three patients were from the same LTCF before being admitted to this hospital. Therefore, medical charts were reviewed and the isolates were submitted for the following laboratory examinations. For comparison, another two imipenem-intermediately resistant P. stuartii isolates were included as controls in genotyping analysis. They were identified from patients of other sources during the study period.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations of the isolates were examined by the BD PHOENIX automated microbiology system (Beckton Dickinson Diagnostic Systems, Sparks, MD, USA). Antimicrobial agents tested were β-lactams (cefpime, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, imipenem, meropenem, piperacillin, and piperacillin/tazobactam), aminoglycoside (amikacin), fluoroquinolone (levofloxacin), phenicol (chloramphenicol), and trimethoprim-sulfamethoxazole. All results were interpreted according to the criteria suggested by the Clinical and Laboratory Standards Institute.

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Genotyping analysis

To reveal the genetic relationship of the isolates, pulsed-field gel electrophoresis was performed using a method described previously. Briefly, bacterial suspensions were embedded in plugs and treated with Smal. The resulting macrorestriction DNA fragments were then subjected to electrophoresis using a CHEF Mapper XA System (Bio-Rad Laboratories, Hercules, CA, USA). An autoalgorithm mode was selected with the running molecular weights ranging from 20 kb to 300 kb. The criteria proposed by Tenover et al. were used for the interpretation of the resulting patterns. Isolates showing identical banding patterns were designated as the same pulsotypes. Those which varied by one to three bands were defined as subtypes of the same pulsotypes. Isolates which differed by four or more bands were considered as different pulsotypes.

Mechanisms of antimicrobial resistance

The presence of genes encoding for extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases was examined by polymerase chain reactions (PCRs) followed by sequence analysis. For fluoroquinolone resistance, PCR/sequencing was also used to detect the mutations present in the quinolone resistance-determining regions among the chromosomal gyrA, gyrB, parC, and parE genes as well as the existence of plasmid-mediated fluoroquinolone resistance genes, such as aac(6')-Ib-cr, qnr, qep, and qnrS. The two major outer membrane proteins, OmpPst1 and OmpPst2, of P. stuartii were also analyzed by PCR/sequencing to reveal the presence of mutations.

Plasmid analysis followed by Southern hybridization was used to examine the location of various resistance genes. Conjugation experiments were performed by using Escherichia coli J53 as the recipient to reveal whether the resistance plasmids are self-transferable. Replicon typing was performed to delineate the genetic correlation of the isolates.

Results

Clinical characteristics of the three patients are listed in Table 1. They were all male patients and aged over 60 years old. Various underlying diseases had led to a bedridden or immunocompromised status among these patients. Patient P1 was admitted to this hospital 14 months earlier than P2, whereas P2 was hospitalized 37 days earlier than P3. The specimens of P2 and P3 were obtained on the respective admission dates, whereas P1 was obtained 4 days after hospitalization. Various antibiotics had been used among these patients in the 3 months prior to the hospitalization, but only patient P3 had used imipenem and levofloxacin. Several last-line antibiotics were used to treat the P. stuartii infection. All patients recovered and were discharged.

Results of the laboratory investigation are shown in Table 2. Among the five groups of antibiotics tested, only aminoglycoside (amikacin) remained susceptible. For β-lactam antibiotics, the isolates were susceptible only to meropenem, ceftazidime (2 in the susceptible-dose dependent category), and piperacillin-tazobactam (n = 3). All isolates showed intermediate resistance to imipenem (minimum inhibitory concentration, 2 μg/mL). Pulsed-field gel electrophoresis analysis revealed that isolates P1 and P3B/P3U were closely related. The other isolates, P2 and the two control isolates demonstrated different pulsotypes (Figure 1).

Various resistance genes were identified among the isolates (Table 2). Several genes, such as blaqvy-2, qnrD1, and qnrS1, were present on plasmids and were associated with the resistance to the third-generation cephalosporins and fluoroquinolones. The plasmids carrying these resistance genes were of different sizes, but all belonged to the IncA/C family. Despite several trials, we were not able to demonstrate the self-transferability of the plasmids. None of the known carbapenemase genes was found.

Various point mutations and insertion/deletion changes were found in one of the major outer membrane protein genes, ompPst1 (data not shown). Amino acid sequences of the other outer membrane protein gene, ompPst2, were identical with those of the reference strain P. stuartii ATCC 29914.

A single point mutation was found in the chromosomal gyrA (codon 83, AGC to ATC) and parC (codon 84, AGT to ATT) genes, both leading to the amino acid change from serine to isoleucine (Table 2). The changes were also related to the observed fluoroquinolone resistance among the isolates.

Discussion

Despite the close temporal and spatial correlation, genotyping analysis revealed that isolates from patients P2 and

<table>
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<th>Clinical characteristics of the patients.</th>
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<td>Patient</td>
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<td>P1</td>
<td>67</td>
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<td>P2</td>
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<td>P3</td>
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P3 were not genetically related. However, another isolate from patient P1, although identified more than 1 year ago, showed an unexpectedly similar pulsed-field gel electrophoresis pattern to those from patient P3. Even though we were not able to conduct environmental surveillance in the LTCF, it is highly suspected that the bacterium may have inhabited in the LTCF and caused opportunistic infections.

Previous reports indicated that *P. stuartii* could reside in a hospital environment, such as hand-washing sinks and tracheal aspirators/probes/tubes, and cause outbreaks of nosocomial infections. In fact, due to the characteristics of LTCF residents, as well as the strategy of nursing care in such institutions, a wide spectrum of MDROs has been found to colonize the environment of LTCFs, causing cross-transmission or outbreaks of infections with high mortality.

In Taiwan, similar situations also have been reported for other MDROs, but never in *P. stuartii*.26,27 Urinary tract infection could be associated with as high as more than 40% of the LTCF residents.28 Asymptomatic bacteriuria could be even higher in almost 60% of such populations, and 16.2% of the cases could be associated with *P. stuartii*.29 The present study further demonstrated that genetically closely-related *P. stuartii* could cause infection in different residents even in more than 1 year apart. Actually, multidrug-resistant *P. stuartii* has been reported to cause cross-transmission and infection for more than 4 years in one single hospital. Another report also demonstrated a 2-year outbreak in a burn unit caused by multidrug-resistant *P. stuartii*.26 In the present study, it remains unknown whether *P. stuartii* caused asymptomatic

![Figure 1. Pulsed-field gel electrophoresis of the four isolates of *Providencia stuartii* (P1, P2, P3B, and P3U) studied and two control isolates (C1 and C2). M, lambda DNA concatamer standard.](image-url)
bacteriuria in other residents or infections that were treated in other hospitals. However, as LTCFs or infections that are usually vulnerable to infections, surveillance of MDROs and infection control interventions that have been established in acute-care hospitals to control infections by resistant organisms are apparently as essential in LTCFs. In fact, the Association for Professionals in Infection Control and Epidemiology and the Society for Healthcare Epidemiology of America have established guidelines for the infection prevention and control in LTCFs.

Governments in other countries, such as the United Kingdom, also have provided sources of information on this regard. However, there are still some countries, including Taiwan, that have not yet established a system. It is apparently an important requisite that special care has to be implemented in LTCFs to prevent the formation of infection reservoirs and spread of MDROs among LTCFs.

*P. stuartii* is known to possess chromosomal AmpC genes and is therefore naturally resistant to aminopenicillins and narrow-spectrum cephalosporins. Nevertheless, the four isolates studied herein still acquired another plasmid-mediated AmpC gene, *bla*_{CMY-2}, and thereby broadened the resistance spectrum to include the third-generation cephalosporins. The situation is similar to some recent reports published elsewhere. Furthermore, plasmid-mediated fluoroquinolone resistance genes, *qnrD1* and *aac(6’)-Ib-cr*, were identified, and combined with the point mutations in the chromosomal *gyrA* and *parC* genes, have resulted in the observed high-level resistance to fluoroquinolones in our isolates. Similar problems have only recently been reported. In fact, *qnrD1* and *aac(6’)-Ib-cr* were only sporadically found in previous reports from Taiwan.

The coexistence of these fluoroquinolone resistance genes and the AmpC gene, *bla*_{CMY-2}, on the same plasmids, as found among the study isolates herein, may represent another potential mechanism for the dissemination of resistance genes and thus the increase of MDROs in LTCFs.

Clinically, empirical therapy by carbapenems is suggested for life-threatening infections or those associated with multidrug-resistant Gram-negative organisms, including *P. stuartii*. Patients P2 and P3 studied herein were indeed successfully cured with doripenem. However, the four isolates studied all expressed low-level resistance to imipenem. Some amino acid changes were revealed in the major outer membrane protein OmpPst1. Previous reports from Taiwan indicated that the major mechanism for the development of CRE was the production of extended-spectrum β-lactamase or AmpC-type enzymes plus mutations in the outer membrane proteins. Therefore, if the factors associated with the currently observed mutational changes persisted, the outer membrane proteins of these imipenem-intermediately resistant *P. stuartii* may continue to mutate, eventually leading to full resistance to all carbapenems. Since *P. stuartii* is naturally resistant to colistin and tigecycline, the two antimicrobial agents frequently used for CRE infection, treatment for *P. stuartii* showing full resistance to carbapenems will become a big challenge. Furthermore, compared to meropenem, imipenem was shown to have a lower level of permeation through the major outer membrane protein OmpPst1 in *P. stuartii*. The observation was well correlated with the lower antibiotic susceptibility of the bacterium to imipenem than to meropenem. The actual underlying mechanism for this relative difference in susceptibilities to the two carbapenems remains to be identified. However, the use of imipenem is likely to provide more selection pressure and hence induce more mutational changes in OmpPst1, leading to higher resistance levels to imipenem, or even to other carbapenems. Therefore, even if antimicrobial susceptibility testing does not demonstrate a resistance result, imipenem is apparently not appropriate for the treatment of *P. stuartii* infection.

In conclusion, the present study demonstrated the possibility for a long-term habitation of multidrug-resistant *P. stuartii* in one LTCF. Similar situations may be found in other multidrug-resistant microbial pathogens. LTCFs appear to be a convenient reservoir for antimicrobial resistance organisms. Surveillance of MDROs and infection control intervention are both required for LTCFs to reduce the infection loads associated with multidrug-resistant bacteria.

**Conflicts of interest**

All authors have no conflicts of interest.

**References**


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