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NOTE

Multidrug-Resistant *Providencia* Isolates Carrying *bla*$_{PER-1}$, *bla*$_{VIM-2}$, and *armA*

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During May to July 2004, three strains of *Providencia* spp. with multidrug-resistance (MDR) were isolated from urinary specimen of three patients hospitalized with a same hospital room. By PCR analysis, all three strains have been found to carry both VIM-2 type metallo-β-lactamase gene and PER-1 type extended-spectrum β-lactamase gene. One out of three strains carried additional resistance gene, *armA*, 16S rRNA methylase gene responsible for high level resistance to aminoglycosides. To our knowledge, this is the first report on the identification of *Providencia* spp. simultaneously carrying *bla*$_{VIM-2}$, *bla*$_{PER-1}$, and *armA* genes.

Keywords: *Providencia*, metallo-β-lactamase, extended-spectrum β-lactamase, 16S rRNA methylase

The genus *Providencia* is a member of the tribe *Proteeae,* with the genus *Proteus* and *Morganella*. *Providencia* consists of four species, *P. alcalifaciens*, *P. stuartii*, *P. rettgeri*, and *P. rustigianii*; one of which, *P. rettgeri* was classified previously in the genus *Proteus*. *Providencia* infections are almost exclusively nosocomial and patients with long-term indwelling urinary catheters are prone to developing bladder colonization with these organisms and this colonization provides a reservoir of organisms for outbreaks in long-term care facilities (Peter, 1992).

Recently, three strains of *Providencia* spp. resistant to multiple antibiotics were isolated from three patients hospitalized in a tertiary hospital in Cheongju, Republic of Korea. All strains were isolated from the catheterized urine of patients hospitalized in the neurosurgical intensive care unit during the same period. In this study, we identified antimicrobial resistance genes responsible for the resistance to multiple drugs of these strains and performed pulsed-field gel electrophoresis (PFGE) to reveal the genomic diversity of these strains.

Antimicrobial susceptibility testing and determination of the minimal inhibitory concentration (MIC) were performed by the broth microdilution method recommended by the CLSI (formerly NCCLS) (National Committee for Clinical Laboratory Standards, 2003). Detection of genes for *bla*$_{PER-1}$, *bla*$_{IMP-1}$, *bla*$_{VIM-1}$, and *bla*$_{VIM-2}$ was performed by PCR using previously reported primers and methods (Lee *et al.*, 2003; Poirel *et al.*, 2005). The *armA* and *rmtB*, 16S rRNA methylase genes confer high-level resistance to aminoglycosides, were detected by PCR using previously reported primers and methods (Yan *et al.*, 2004). PFGE of genomic DNAs were performed by the previously reported method (Gautom, 1997).

Among three isolates of *Providencia* spp., two strains were identified as *P. rettgeri* and one was *P. stuartii.* The three strains showed high-level resistance to the following antimicrobial agents; ampicillin, cefoxitin, cefotaxime, aztreonam, cefepime, ampicillin/sulbactam, imipenem, meropenem, amikacin, and ciprofloxacin (Table 1). Based on the antimicrobial resistance pattern, the three strains were presumed to produce extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL). By a PCR analysis, both *bla*$_{PER-1-like}$ and *bla*$_{VIM-2-like}$ genes were detected in all three strains and *armA* gene, one of the 16S rRNA methylase genes confer high-level resistance to aminoglycosides, was additionally detected from *P. rettgeri* 1162 strain (Fig. 1 and Table 1). *Not*I digested PFGE patterns of three strains were different from each other, indicating different clonal origin (Fig. 2).

The three patients, from whom MDR *Providencia* was isolated, hospitalized together in the neurosurgical intensive care unit during the same period (May to July, 2004) and they were catheterized with urinary catheter. The underlying disease of the three patients was spinal injury, cerebral infaraction, or subdural hemorrhage, respectively. The patients were suffered from infections of other sites (pneumonia or bed sore) and ceftriaxone was administered for the treatment of the infection. Only one (strain 852) out of three isolates was suspected as a true pathogen because pyuria (>25 WBCs/HPF) was observed in the patient from whom the isolate 852 was obtained. However, microorganisms and leukocytes were not detected anymore after changing of urinary catheter, so the isolate 852 was considered as a colonizer rather than a true pathogen. Other two isolates were also considered as a colonizer or contaminator due to the absence of pyuria. From the culture of specimens obtained from environment, *P. rettgeri* was isolated from the patient's urine container and a tap of a toilet. The isolate showed

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Table 1. Antimicrobial susceptibility and carrying resistant genes of *Providencia* spp.

Isolates	MIC (μg/ml)[a]									Resistant genes		
	AMP	FOX	CTX	AZT	FEP	IMP	MEM	AMK	CIP	*VIM-2*	*PER-1*	*armA*
P. rettgeri 852	>256	128	>256	>256	>512	16	8	512	512	+	+	–
P. rettgeri 1162	>256	128	>256	>256	>512	16	8	>512	512	+	+	+
P. stuartii 130	>256	128	>256	>256	>512	16	8	256	512	+	+	–

[a]Abbreviation: AMP, ampicillin; FOX, cefoxitin; CTX, cefotaxime; AZT, aztreonam; FEP, cefepime; IMP, imipenem; MEM, meropenem; AMK, amikacin; CIP, ciprofloxacin.

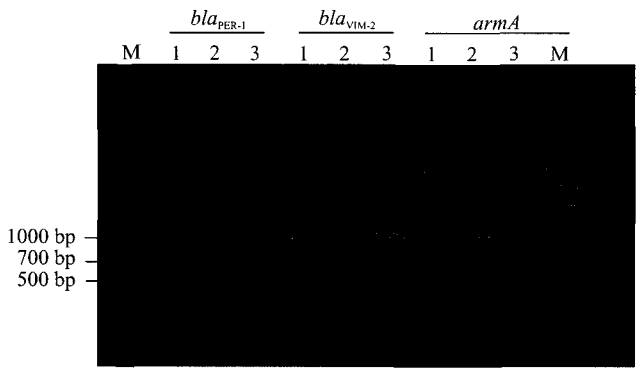

Fig. 1. Detection of bla_{PER-1}, bla_{VIM-2}, and *armA* in *Providencia* spp. by a PCR. M, DNA size marker; lane 1-3, *P. rettgeri* 852, *P. rettgeri* 1162, and *P. stuartii* 130.

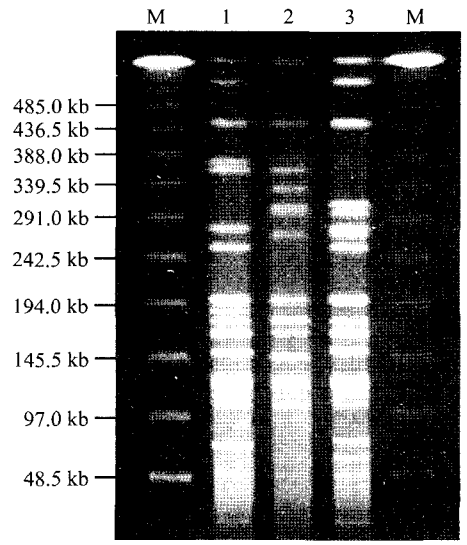

Fig. 2. Pulsed-field gel electrophoresis (PFGE) analysis of *Providencia* spp.. *Not*I-digested genomic DNA was resolved on 1% agarose gel by PFGE. M, DNA size marker; lane 1-3, *P. rettgeri* 852, *P. rettgeri* 1162, and *P. stuartii* 130.

similar resistant pattern with clinical isolates and revealed positive results from the PCR for the detection of bla_{PER-1} and bla_{VIM-2}. As the results of efforts such as disinfection, perfect separation of urine container and changing of urinary catheter, further isolation of MDR *Providencia* spp. was not observed with an exception of MDR *P. rettgeri* one more time from one patient after three weeks.

To our knowledge, this is the first observation of *Providencia* spp. isolate simultaneously carrying bla_{PER-1}, bla_{VIM-2}, and *armA* genes. Until this time, only one isolate simultaneously carrying bla_{PER-1} and bla_{VIM-2} genes has been reported in *P. aeruginosa* (Docquier *et al.*, 2001) but isolate simultaneously carrying bla_{PER-1}, bla_{VIM-2}, and *armA* genes has never been reported.

The PER-1 enzyme is a class A type ESBL and confers resistance to penicillin, cefotaxime, ceftibuten, ceftazidime, and monobactams (aztreonam) but spares resistance to carbapenems and cephamycins. This enzyme was firstly detected in 1993 in an isolate of *P. aeruginosa* from a Turkish patient in France (Nordmann *et al.*, 1993) and has also been detected from cefepime-resistant *Acinetobacter* spp. in Korean hospitals (Yong *et al.*, 2003). The bla_{PER-1} gene is widespread in *Acinetobacter* spp., *Salmonella enterica* seovar Typhimurium in Turkey and has also been detected in *Providencia* spp. in that country (Vahaboglü *et al.*, 1996, 1997; Bahar *et al.*, 2004).

The VIM-2 enzyme was first detected in a *P. aeruginosa* strain isolated in Marseilles, France, in 1996 (Poirel *et al.*, 2000) and confers resistance to not only carbapenems but also virtually all β-lactams with the exception of monobactams (Poirel *et al.*, 2000, 2001). In Korea, VIM-2 is the most frequently detected MBL since the first identification of VIM-2 in *P. aeruginosa* isolated in 1995 (Lee *et al.*, 2002). This enzyme has been reported in isolates of other species such as *P. putida*, *Acinetobacter* spp., *S. marcescens*, *E. cloacae*, and *A. xylosoxidans* in Korea (Lee *et al.*, 2002; Yum *et al.*, 2002a, 2002b; Jeong *et al.*, 2003; Shin *et al.*, 2005).

The *armA* gene was firstly detected from a *K. pneumoniae* isolate in France in 2003 and the clinical isolates carrying *armA* gene confer high-level resistance to aminoglycosides including amikacin and arbekacin (Mingeot-Leclercq *et al.*, 1999; Galimand *et al.*, 2003; Lee *et al.*, 2006). Recently, Yan *et al.* (2004) and Lee *et al.* (2006) reported dissemination of *armA* gene in amikacin-resistant *E. coli*, *K. pneumoniae*, and *Acinetobacter* spp. in Taiwan and Korea, respectively. They also found that the *armA* gene is located on a plasmid with genes encoding CTX-M type ESBL or DHA-1 plasmid-mediated AmpC type β-lactamase.

In Korean hospitals, bla_{PER-1}, bla_{VIM-2}, and *armA* genes were detected separately from clinical isolates (Lee *et al.*, 2002, 2006; Yong *et al.*, 2003) and it has been worried about the emergence of new threatening combinations of the resistance determinants. In this study, two *Providencia* isolates carrying both bla_{PER-1} and bla_{VIM-2} genes and one isolate simultaneously carrying bla_{PER-1}, bla_{VIM-2}, and *armA*

genes were identified. The recruitment of bla_{PER-1}, bla_{VIM-2}, and *armA* genes within a single strain can determine a resistance phenotype virtually almost of the available antimicrobial agents such as β-lactams, monobactams, carbapenems, and aminoglycosides, which can be detrimental. Therefore, it should be developed a strategy for effective control and preventing dissemination of such strains.

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