Prevalence of β-lactamase classes A, C, and D among clinical isolates of *Pseudomonas aeruginosa* from a tertiary-level hospital in Bangkok, Thailand

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ABSTRACT. *Pseudomonas aeruginosa* is one of the most important causes of nosocomial infection and it has increasing resistance to many antimicrobial agents. β-lactamase production is the most frequent mechanism for β-lactam resistance in *P. aeruginosa*. We evaluated the prevalence of β-lactamase genes in *P. aeruginosa* for classes A, C, and D by polymerase chain reaction, and investigated clonal diversity by pulsed-field gel electrophoresis (PFGE). We used the disk diffusion

method to test 118 non-duplicate clinical isolates of *P. aeruginosa* for antimicrobial susceptibility. We identified 51 isolates (43.22%) as multidrug-resistant *P. aeruginosa*, approximately 44.91% of which were resistant to ceftazidime. β-lactamase genes were found in 80 isolates of *P. aeruginosa* (67.80%). The genes that encode VEB-1, AmpC, and OXA-10 were detected in 9 (7.62%), 75 (63.56%), and 18 (15.25%) of these isolates, respectively. The genes that encode PER-1, CTX-M, TEM-1 and derivatives, and SHV-1 were not found in any of the *P. aeruginosa* isolates. We identified 29 different pulsotypes by PFGE. Two predominant pulsotypes were found. In pulsotype 1, OXA-10, which was co-produced with the AmpC gene, was predominant. Moreover, VEB-1-producing strains were found to be scattered in many pulsotypes, and AmpC-producing strains showed high pulsotype diversity. The prevalence of β-lactamase genes in *P. aeruginosa* was represented by the genetic heterogeneity of OXA-10, AmpC, and VEB-1. The predominant clone of *P. aeruginosa* clinical isolates was OXA-10. This raises concern about oxacillinases among *P. aeruginosa* clinical isolates.

Key words: β-lactamase; Polymerase chain reaction; *Pseudomonas aeruginosa*; Pulsed-field gel electrophoresis

INTRODUCTION

Pseudomonas aeruginosa, a non-fermentative Gram-negative bacterium, is an opportunistic pathogen that can cause nosocomial infection. It can tolerate diverse environmental conditions and can be resistant to many antimicrobial agents (Todar, 2014). In Thailand, the rate of resistance to ceftazidime increased from 23.00 to 27.80% between 2000 and 2013 (National Antimicrobial Resistance Surveillance Center, 2014). The SENTRY Antimicrobial Surveillance Program, conducted between 2009 and 2012, revealed that *P. aeruginosa* showed reduced susceptibility to most antimicrobial agents tested, and the resistance rates to ceftazidime were 16.10% in the USA and 24.00% in Europe and the Mediterranean region (Sader et al., 2014). In Asia, specifically in China, there was 51.60% ceftazidime resistance in *P. aeruginosa* isolates (Li et al., 2015). The mechanisms that *P. aeruginosa* and other bacteria use to destroy antimicrobial agents include: 1) reducing the permeability of the cell membrane; 2) using the efflux pump system; 3) changing the structure of the target drug molecule; and 4) producing an enzyme such as β-lactamase to destroy the antimicrobial agent, which is the most relevant mechanism. β-lactamase enzymes can be divided into the following four Ambler classes: A, B, C, and D (Livermore, 2002; Shen and Fang, 2015). Class B comprises metallo-β-lactamases, which use zinc for the facility reaction and can be inhibited by ethylenediaminetetraacetic acid (EDTA), whereas classes A, C, and D comprise serine β-lactamases, which can be resistant to many classes of cephalosporin and oxacillin that use antipseudomonal β-lactam drugs. Class A is the most diverse class; it comprises enzymes that can be inhibited by β-lactamase inhibitors such as clavulanic acid, tazobactam, and sulbactam. Class A β-lactamases are encoded by many genes, such as those that encode VEB, PER, CTX, SHV, and TEM (Bush and Jacoby,

2010). Class C and D β-lactamases can be resistant to cephamycin (a type of cephalosporin) and to oxacillin, respectively. The enzymes in these classes cannot be inhibited by β-lactamase inhibitors. There are many variants of the genes that encode class D β-lactamases, such as OXA-1, OXA-2, and OXA-10; however, OXA-10 is the prototype gene and is resistant to oxacillin and cephalosporin (Poirel et al., 2010).

For phenotypic screening, we used a combined disk test for class A and an AmpC disk test for class C; no guidelines or reference methods have been provided for *P. aeruginosa* (Livermore and Brown, 2001; Black et al., 2005). However, the prevalence of class A, C, and D β-lactamase genes alone is not sufficient to explain the distribution and genetic relationships of *P. aeruginosa*. Pulsed-field gel electrophoresis (PFGE) is the reference method or "gold standard" for epidemiological studies. This method is similar in principle to normal agarose electrophoresis, but it can be used to separate DNA molecules that are larger than 20 kb. It uses a pulsed alternating voltage gradient that is switched between three directions: one from the central axis of the gel and the other two from 60° either side of the axis. This is effective for large DNA molecules that respond to the change in the electric field more slowly than small molecules. As a result, the small DNA molecules move further than the large ones, and more time is required to separate the entire chromosomal DNA molecule, which was previously cut using restriction enzymes. The results can then be interpreted by comparing the pattern of DNA fragments (Goering, 2010).

The aim of this study was to investigate the β-lactamase genes in *P. aeruginosa* for class A (VEB-1, PER-1, CTX-M, SHV-1, TEM-1 and derivatives), class C (AmpC), and class D (OXA-10) enzymes by polymerase chain reaction (PCR), and to distinguish the pulsotypes of the PCR-positive strains of *P. aeruginosa* by PFGE.

MATERIAL AND METHODS

Bacterial strains

One hundred and eighteen isolates of *P. aeruginosa* were collected between March and April 2009 at the 1200-bed, tertiary-level hospital in Bangkok, Thailand. All strains were frozen and maintained at -80°C in trypticase soy broth with 20% glycerol. The *P. aeruginosa* isolates were selected by simple-random sampling. The list of *P. aeruginosa* isolates from March to April of that year from the study hospital was obtained and individuals were selected using a random-number table (Chernick and Friis, 2003). However, after this step, all chosen bacterial isolates were entirely anonymized and assigned new numbers.

Ethical approval and informed consent was not required by our institutional Ethics Committee because all bacterial isolates were collected, processed, and stored as part of routine diagnosis by the hospital. No patient information was associated with the data. The bacterial isolates were anonymized and an identification number was generated prior to DNA extraction.

Antimicrobial susceptibility test

The antimicrobial susceptibility test for β-lactamase was performed using the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute

(CLSI) guidelines. The antimicrobial agents used were ceftazidime (CAZ), cefepime (FEP), cefoperazone/sulbactam (SCFP), piperacillin/tazobactam (TZP), amikacin (AK), gentamicin (GM), ciprofloxacin (CFX), levofloxacin (LVX), imipenem (IPM), meropenem (MEM), and colistin (CL) (Oxoid, Basingstoke, Hampshire, England), and the results were interpreted using the CLSI 2009 criteria (Barry and Jones, 1988; Clinical and Laboratory Standards Institute, 2009).

PCR for the detection of β-lactamase classes A, C, and D

DNA extraction was performed using the modified boiling method. First, *P. aeruginosa* was cultured on Mueller-Hinton agar (Oxoid) for 24 h. A few colonies of *P. aeruginosa* were suspended in 1 mL Nutrient Broth (Oxoid), and the turbidity was adjusted to McFarland standard number 2. The adjusted suspension was boiled at 95°-100°C for 10 min, and then centrifuged at 12,000 *g* at 4°C for 10 min. The supernatant (500 µL) was transferred to a new microfuge tube; the DNA was precipitated using absolute ethanol at -20°C for 10 min and centrifuged at 12,000 *g* at 4°C for 10 min. The pelleted DNA was washed with 70% ethanol and centrifuged at 12,000 *g* at 4°C for 2 min. The pellet was washed three times. The sediment was air-dried, and the DNA was dissolved in 50 µL 1X TE buffer, pH 8.0.

The PCR for the detection of class A, C, and D β-lactamases was performed for all of the clinical isolates in 25-µL reaction volumes using a typical PCR protocol containing 1X Taq DNA polymerase buffer (Thermo Scientific, Waltham, MA, USA), 0.2 mM each dNTP (Promega, Madison, WI, USA), 0.5 µM each primer (IDT, Singapore; Table 1), 1.5 mM $MgCl_2$ (Thermo Scientific), 1 U Taq DNA polymerase (Thermo Scientific), and 100-500 ng template DNA. The amplification program was as follows: an initial denaturation at 95°C for 3 min; 30 cycles of denaturation at 95°C for 30 s, annealing for 30 s (temperatures as shown in Table 1), extension at 72°C for 1 min; and a final extension at 72°C for 5 min. The PCR was performed in a model MJ Mini PTC-1148 thermocycler (Bio-Rad, Hercules, CA, USA), and then the PCR products were used for electrophoresis on 1.5% agarose gel that contained 1X SYBR safe DNA strain (Invitrogen, Carlsbad, CA, USA), run at 80 V for 40 min. The PCR product bands were detected using Chemidoc XRS (Bio-Rad).

Table 1. Sequences and conditions used for primers in this study.

Gene		Primer (5'-3')	Annealing temperature (°C)	Product size (bp)	Reference
Class A	VEB-1	F: cgacttccatttcccgatgc R: ggactctgcaacaaatacgc	58.60	650	(Lin et al., 2012)
	PER-1	F: atgaatgtcattataaaagc R: aatttgggcttagggcagaa	52.30	920	(Lin et al., 2012)
	CTX-M	F: cgctttgcgatgtgcag R: accgcgatatcgttggt	49.50	550	(Lin et al., 2012)
	TEM-1 and derivatives	F: ataaaattcttgaagacgaaa R: gacagttaccaatgcttaatca	43.10	1079	(Lee et al., 2005)
	SHV-1	F: tcagcgaaaaacaccttg R: tcccgcagataaatcacca	60.00	475	(Yuan et al., 1998)
Class C	AmpC	F: atgcagccaacgacaaagg R: cgccctcgcgagcgcgcttc	57.60	1243	(Lin et al., 2012)
Class D	Oxa-10	F: tctttcgagtacggcattagc R: ccaatgatgccctcactttcc	60.22	760	(Lin et al., 2012)

PFGE

The molecular typing of *P. aeruginosa* isolates carrying β-lactamase genes was performed by PFGE according to the manufacturer protocol (Bio-Rad), with some modifications (Selim et al., 2015). *Spe*I-HF (15 U; New England Biolabs, Ipswich, MA, USA) was used to digest the chromosomal DNA embedded on an agarose plug at 37°C for 18 h. The digested plug was then electrophoresed on 1% Certified Megabase Agarose (Bio-Rad) in 0.5X Tris-borate-EDTA buffer using a contour-clamped homogeneous electric field DRII apparatus (Bio-Rad) at 6 V/cm, at a temperature of 12°C, an angle of 120°, and 1-50-s pulses over a period of 24 h. The gel was stained with 0.5 µg/mL ethidium bromide to visualize the DNA band profiles, and analyzed with the trial license (30 days) of the Bionumerics software for Windows version 7.5 (Applied Maths, Kortrijk, Belgium). The PFGE patterns were compared using the UPGMA (unweighted pair group method with arithmetic averages) clustering method (Dice coefficient optimization of 1.5% and tolerance in band position of 1.5%). Isolates were considered to be the same pulsotype (PT) if the relatedness was ≥80%, with ≤6 band differences (Tenover et al., 1995).

RESULTS

Antimicrobial susceptibility test for β-lactamase

The antimicrobial susceptibility tests of the 118 clinical isolates showed that all the strains were susceptible to CL. The fluoroquinolone drugs LVX and CFX had the highest resistance rates of 49.15% (58/118) and 48.30% (57/118), respectively. These were followed by CAZ, SCFP, TZP, MEM, IPM, FEP, GM, and AK, which had resistance rates of 44.91% (53/118), 38.98% (46/118), 36.44% (43/118), 33.05% (39/118), 31.36% (37/118), 28.81% (34/118), 25.42% (30/118), and 18.64% (22/118), respectively (Figure 1).
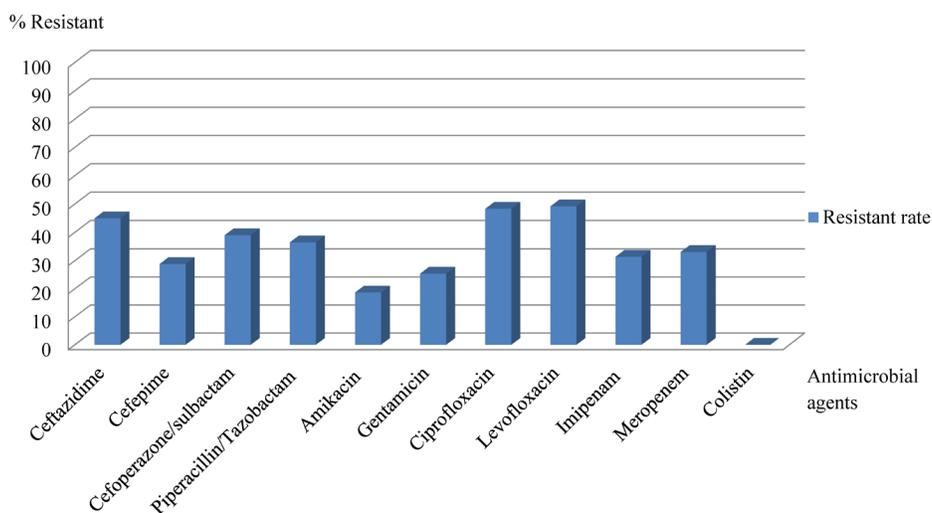

Figure 1. Resistance rate of 118 strains of *Pseudomonas aeruginosa*.

The 118 *P. aeruginosa* isolates had 18 different antibiogram patterns (Table 2). Twenty isolates were resistant to all five of the antibiotic groups used (except CL) and were categorized into a group called antibiotic resistance pattern (ARP) 1. Nineteen were resistant to four of the antibiotic groups used (ARP2, susceptible to carbapenems; ARP3, susceptible to aminoglycoside; and ARP4, susceptible to β-lactam/inhibitor). Twelve were resistant to three of the antibiotic groups used (ARP5-9). Fifty-one isolates (43.22%), ARP1-9, were defined as multidrug-resistant *P. aeruginosa* (MDR-PA) using the criterion that they were resistant to three or more antibiotic groups (Magiorakos et al., 2012). Sixty-seven isolates were non-MDR-PA. Thirteen were resistant to two of the antibiotic groups used (ARP10, resistant to β-lactam and β-lactam/inhibitor; ARP11, resistant to β-lactam and fluoroquinolones; ARP12, resistant to β-lactam/inhibitor and carbapenems; ARP13, resistant to aminoglycoside and fluoroquinolones; ARP14, resistant to fluoroquinolones and carbapenems). Seven were resistant to one of the antibiotic groups used (ARP15, resistant to β-lactam/inhibitor; ARP16, resistant to fluoroquinolones; ARP17, resistant to carbapenems), and a large group of 47 remained susceptible to all antibiotic groups (ARP18).

Table 2. Antibiotic resistance patterns of 118 clinical isolates of *Pseudomonas aeruginosa*.

Antibiotic resistance patterns	Antibiotics group						No. of strains
	β-lactam	β-lactam/inhibitor	Aminoglycoside	Fluoroquinolones	Carbapenems	Polymyxin	
1	R	R	R	R	R	S	20
2	R	R	R	R	S	S	3
3	R	R	S	R	R	S	13
4	R	S	R	R	R	S	3
5	R	R	S	R	S	S	6
6	S	R	S	R	R	S	3
7	R	R	S	S	R	S	1
8	R	S	R	S	R	S	1
9	R	S	S	R	R	S	1
10	R	R	S	S	S	S	5
11	R	S	S	R	S	S	2
12	S	R	S	S	R	S	2
13	S	S	R	R	S	S	3
14	S	S	S	R	R	S	1
15	R	S	S	S	S	S	1
16	S	S	S	R	S	S	3
17	S	S	S	S	R	S	3
18	S	S	S	S	S	S	47

S = susceptible, R = resistant.

PCR for the detection of β-lactamase classes A, C, and D

The PCR method was used to investigate β-lactamase genes in the 118 isolates of *P. aeruginosa* for seven genes, including the following: β-lactamase class A, including VEB-1, PER-1, CTX-M, TEM-1 and derivatives, and SHV-1; β-lactamase class C, including AmpC; and β-lactamase class D, including OXA-10. Eighty (67.80%) isolates were positive. β-lactamase genes VEB-1, AmpC, and OXA-10 were detected in 9 (7.62%), 75 (63.56%) and 18 (15.25%), respectively. Among the 18 OXA-10-producing *P. aeruginosa* isolates, all of the strains carried other genes, including 4 (3.39%) with VEB-1, 13 (11.01%) with AmpC, and 1 (0.85%) with VEB-1 and AmpC. Three isolates (2.54%) harbored both VEB-1 and AmpC. PER-1, CTX-M, TEM-1 and derivatives, and SHV-1 genes were not found in any of the *P. aeruginosa* isolates (Table 3).

Of the 9 VEB-1-producing isolates, the strains that were MDR-PA had ARPs of 1, 2, 3, 4, and 9 and 100% resistance to the β-lactam drugs (CAZ and FEP). In 13 of both the AmpC- and OXA-10-carrying isolates, all of the strains were MDR-PA except for one that was ARP18 and was at least 84% resistant to five of the antibiotic groups used (except CL). However, the strains carrying only the AmpC gene were MDR-PA (41.38%) and non-MDR (58.62%).

Table 3. Distribution of β-lactamase genes, antibiotic resistance profiles, and pulsotypes among the *Pseudomonas aeruginosa* clinical isolates.

β-lactamase genes		No. of isolates (%)	No. of resistant isolates (%)						Antibiotic resistance pattern	Pulsotype
			β-lactam	β-lactam/ inhibitor	Amino- glycoside	Fluoro- quinolones	Carbapenem	Polymyxin		
Single gene	VEB-1 only	1 (1/118, 0.85%)	1 (100%)	0 (0.00%)	0 (0.00%)	1 (100%)	1 (100%)	0 (0.00%)	9	PT11
	AmpC only	58 (58/118, 49.15%)	24 (41.38%)	24 (41.38%)	7 (12.07%)	27 (46.55%)	21 (36.20%)	0 (0.00%)	1(6), 3(9), 5(6), 6(2), 7, 11(2), 13, 14, 17(2), 18(28)	PT1(6), PT2(17), PT3(2), PT4(3), PT5(2), PT6(3), PT7(2), PT8(1), PT9(2), PT10(2), PT11(1), PT12(1), PT13(2), PT14, PT15, PT16, PT17, PT18, PT19, PT20, PT21, PT22, PT24, PT25, PT26, PT28, PT29
Multiple genes	VEB-1 + AmpC	3 (3/118, 2.54%)	3 (100%)	2 (66.67%)	2 (66.67%)	3 (100%)	3 (100%)	0 (0.00%)	1, 3, 4	PT3, PT4, PT27
	VEB-1 + Oxa-10	4 (4/118, 3.39%)	4 (100%)	3 (75.00%)	4 (100%)	4 (100%)	2 (50.00%)	0 (0.00%)	1, 2(2), 4	PT3, PT5, PT12, PT23
	AmpC + Oxa-10	13 (13/118, 11.01%)	12 (92.31%)	11 (84.61%)	12 (92.31%)	11 (84.61%)	12 (92.31%)	0 (0.00%)	1(11), 8, 18	PT1 (13)
	VEB-1 + AmpC + Oxa-10	1 (1/118, 0.85%)	1 (100%)	0 (0.00%)	1 (100%)	1 (100%)	1 (100%)	0 (0.00%)	4	PT8
Negative		38 (38/118, 32.2%)	11 (28.95%)	13 (34.21%)	4 (10.52%)	11 (28.95%)	8 (21.05%)	0 (0.00%)	1, 2, 3(3), 6, 10(5), 12(2), 13(2), 15, 16(3), 17, 18(18)	

PFGE

Figure 2 shows a dendrogram of 80 of the strains (PCR-positive for AmpC, VEB-1, and OXA-10 genes); it shows that PFGE was capable of determining the type of 78 (97.50%) of the isolates, whereas two of the isolates produced smeared bands. The fragment band patterns from 250-800 kb were detected after the genomic DNA had been digested using *Spe*I-HF. Twenty-nine different PTs were identified, and two major PTs (PT1, PT2) applied to 19 and 17 isolates, respectively. Four of the isolates belonged to two smaller PTs, three of the isolates belonged to another two PTs, two of the isolates belonged to seven PTs, and 16 of the isolates belonged to 16 unique PTs. One large PT (PT1), including 19 of the isolates, was all MDR-PA except for one strain. For PT2, which included 17 of the isolates, 10 of the isolates (10/17, 58.82%) were MDR-PA. Thirteen (68.42%) of the isolates carrying OXA-10, which was co-produced with the AmpC gene, were noted predominantly in PT1. Nine of the VEB-1-producing strains were clustered into the following PTs: PT3 (two isolates), PT4, PT5, PT11, PT12 and a unique PT (PT23 and PT27). The AmpC-producing strains showed diverse PTs (PT28) among 75 of the isolates. The major PT (PT1) comprised 19 of the isolates; the small one, PT2, comprised 17 of the isolates. Of the other PTs, one PT had four isolates, two PTs had three isolates, six PTs had two isolates, and each of the 17 PTs had one isolate.
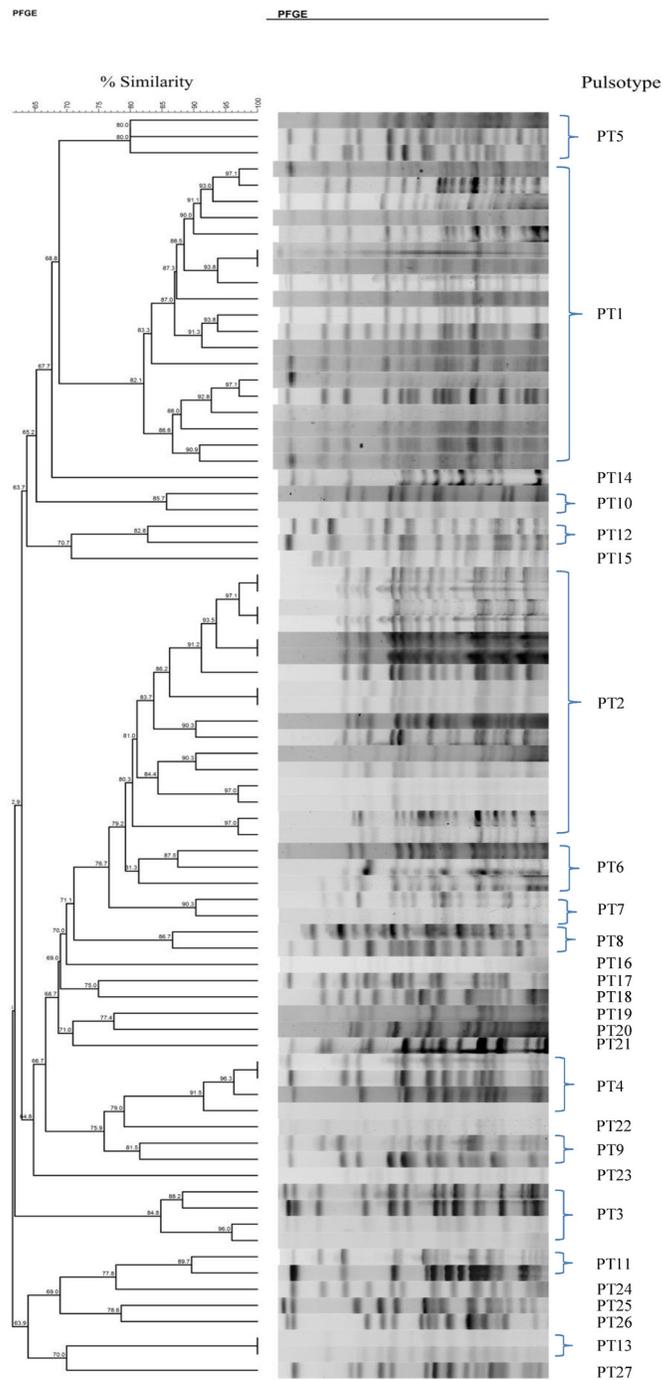
Figure 2. Dendrogram of 80 PCR-positive strains of *Pseudomonas aeruginosa* generated by BioNumeric V.7.5.

DISCUSSION

P. aeruginosa is an increasing healthcare problem worldwide and is known as a multidrug-resistant organism. In the present study, CAZ had a high resistance rate of approximately 44.91% (53/118), in agreement with the results obtained by Woodford et al. (2008) and Li et al. (2015), who found CAZ resistance rates of 47.00 and 51.60%, respectively. However, studies in the USA, Europe and the Mediterranean region (Sader et al., 2014), and in Turkey (Vahaboglu et al., 1997) revealed lower rates of CAZ resistance (16.10-24.00 and 28.00%, respectively). Other reports have shown a higher rate of CAZ resistance, such as in Iran (100.0%) (Mirsalehian et al., 2010). Among the 118 *P. aeruginosa* isolates investigated in this study, 51 (43.22%) were defined as MDR-PA, which was similar to the results of a study performed in China (37.90%) (Li et al., 2015). However, this number was lower than that reported by Vaez et al. (2015) and Mirsalehian et al. (2010) (51.80 and 87.05%, respectively). All the *P. aeruginosa* isolates in this study were susceptible to polymyxin E (CL), in agreement with other study (Woodford et al., 2008).

In the present study, for β-lactamase genes, 80 of the isolates were identified by PCR as positive for VEB-1, OXA-10, and AmpC genes. PER-1, CTX-M, SHV-1, and TEM-1 and derivatives were not detected. A previous study reported that SHV, TEM, and CTX-M β-lactamase genes are very rarely isolated from *P. aeruginosa* (Potron et al., 2015). The PER-1 gene was found in Turkey and Iran as well as in some countries in Asia, such as China (Vahaboglu et al., 1997; Mirsalehian et al., 2010; Qing et al., 2014).

Because it is an intrinsic gene in *P. aeruginosa*, in this study, the AmpC gene was found to be more highly expressed than the other two genes (63.56%, 75/118) (Lister et al., 2009). In our investigation, we found that VEB-1 had a 7.62% (9/118) prevalence that was similar to that in China (11.4%) (Chen et al., 2015) and in the Middle East, in countries such as Saudi Arabia (10.00%) (Al-Agamy et al., 2012) and Iran (12.35%) (Mirsalehian et al., 2010). These results were in contrast to the studies from Europe, which showed a higher prevalence than in our study (Woodford et al., 2008).

For class D β-lactamase, we found a prevalence of 15.25% (18/118), which was nearly the same rate as in Korea (13.10%) (Lee et al., 2005). However, our data showed a lower prevalence than that reported by Bae et al. (2014) (28.29%), and in some countries in the Middle East, such as Iran (29.41%) (Mirsalehian et al., 2010).

In this study, of the nine isolates of VEB-1-producing *P. aeruginosa*, all of the strains were resistant to the β-lactam drugs (CAZ and FEP). Moreover, in the OXA-10-producing strains, 94.44% (17/18) were resistant to CAZ. These data indicate that a high level of resistance to CAZ could be involved in the presentation of VEB-1 and OXA-10 (Nordmann and Guibert, 1998; Aubert et al., 2001). Whereas those isolates that were AmpC positive only showed a moderate rate of resistance to CAZ (41.38%, 24/58), this was associated with a decreased susceptibility to expanded-spectrum cephalosporins such as CAZ (Rodríguez-Martínez et al., 2009a). All of the OXA-10-producing isolates were co-expressed with VEB-1, AmpC, or both genes. These data suggest that OXA-10 and VEB-1 are encoded by a gene located on a mobile element, such as a class 1 integron (Sanschagrin et al., 1995; Girlich et al., 2002; Strateva and Yordanov, 2009). We found that 32.00% of the class 1 integrons of 75 isolates in a study by Piyakul et al. (2012) were also present in the clinical isolates in the current study. However, this study did not investigate other mechanisms that may be responsible for resistance to β-lactam such as the alteration or loss of porin protein, or the upregulation of efflux systems.

In this investigation, we found that the main OXA-10-producing PT (PT1) was similar to that found in studies in France. Vettoretti et al. (2009) found that OXA-10-positive strains were clustered in one main PT, in agreement with another study performed at a University Hospital in France (Cholley et al., 2010), which presented the OXA-10 derivative predominately in one PT. However, the PT of VEB-1-producing strains did not originate from a single clone, as was found in a similar study from central Thailand (Girlich et al., 2002). Moreover, AmpC-producing strains showed high PT diversity, corresponding with the results reported by Rodríguez-Martínez et al. (2009b), who found a clonal diversity in 25 strains of AmpC-positives with 15 PTs. In this study, clinical isolates were collected from only one hospital for genotyping; more isolates from other hospitals in Bangkok or other parts of Thailand are needed to investigate further the molecular epidemiology of *P. aeruginosa*. Moreover, further studies that compare genotyping methods are required.

CONCLUSION

Nearly half of the *P. aeruginosa* clinical isolates had multidrug resistance, although all of the isolates were susceptible to polymyxin E. Various β-lactamase genes were detected; the majority of which were β-lactamase class C (AmpC) followed by class D (OXA-10), and class A (VEB-1). No PER-1, CTX-M, TEM-1 and derivatives, or SHV-1 genes were found. However, the predominant clone of the isolates presented with OXA-10; this supports the concern about oxacillinases in *P. aeruginosa* clinical isolates.

Conflicts of interest

The authors declare no conflict of interest.

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