MOLECULAR EPIDEMIOLOGY OF *PSEUDOMONAS AERUGINOSA* AT A TERTIARY CARE HOSPITAL, SOUTHERN THAILAND

Phanvasri Saengsuwan[1], Narongdet Kositpantawong[2], Natnicha Ingviya[3] and Kamonnut Singkhamanan [1]

[1]Department of Biomedical Sciences, [2]Department of Internal Medicine, [3]Microbiology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla Province, Thailand.

Abstract. *Pseudomonas aeruginosa* ($n = 112$) isolates from various clinical specimens of hospitalized patients in Songklanagarind Hospital, southern Thailand from February 2012 to January 2013 were analyzed for susceptibility to 10 different antibiotics using a disk diffusion method and genetic relatedness by a pulsed-field gel electrophoretic (PFGE). Half of the strains were from sputum samples mainly of infected patients with cardiovascular disease. *P. aeruginosa* infection is significantly associated with age and length of hospital stay (p-value <0.029). *P. aeruginosa* strains were equally sensitive (75-95%) to the four classes of antibiotics commonly used in Songklanagarind Hospital. Highest rate of resistance was to norfloxacin (43%) and no resistance to colistin was detected. Multidrug- and extensively drug-resistant *P. aeruginosa* (MDR-PA and XDR-PA) constituted 18% and 6%, respectively of the strains. PFGE DNA profiling revealed ≥70 relatedness among the strains, clustering into seven groups (A-G). The predominant group A and E (38 and 32% of typeable strains respectively) contained 21% of MDR-PA and 11% of XDR-PA strains. It was noticeable 40% of MDR and XDR-PA strains were from patients in intensive care unit and medical ward. In conclusion, the study shows the usefulness of PFGE DNA profiling in molecular epidemiological investigation of nosocomial *P. aeruginosa* infection allowing implementation of appropriate transmission control and prevention strategies.

Keywords: *Pseudomonas aeruginosa*, antibiogram, DNA profiling, molecular epidemiology, pulsed-field gel electrophoresis

INTRODUCTION

Pseudomonas aeruginosa is one of the most important microorganisms causing nosocomial infections, mainly in impaired immune patients (Sader *et al*, 2017), and continues to be a serious cause of infection with increased mortality and costs due to prolonged hospitalization (Sader *et al*, 2017). Prevalence of multidrug-resistant *P. aeruginosa* (MDR-PA) is increasing worldwide and poses a serious problem in hospital settings as the spread of these organisms is often difficult to eradicate (Pagani *et al*, 2004). Thus, an understanding of epidemiology of

Correspondence: Kamonnut Singkhamanan, Department of Biomedical Sciences, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
E-mail: skamonnu@medicine.psu.ac.th

nosocomial infections is necessary for effective control as well as prevention measures.

Several molecular genotyping techniques have been effectively employed for epidemiological studies of *P. aeruginosa* infection, such as amplified fragment length polymorphism (AFLP), multi-locus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), repetitive extra-palindromic PCR analysis, and restriction fragment length polymorphic DNA analysis (RFLP) (Grundmann *et al*, 1995), all of which have been shown to be useful in epidemiological studies of *P. aeruginosa* isolates (Maatallah *et al*, 2013).

PFGE is currently accepted as the 'gold standard' method and has proven to be useful in elucidating epidemiology of hospital outbreaks (Grundmann *et al*, 1995, Hu and Manos 2015; Santos-Sanches *et al*, 2015). The method can also be used for monitoring genetic evolution of microorganisms and in identifying the most prevalent strain circulating in a population. Incorporation of molecular typing with traditional hospital epidemiological surveillance have resulted in the reduction in number of nosocomial infections and is cost-effective when there is cooperation among laboratory investigation, epidemiological typing and infection control unit (Spiers *et al*, 2000).

Molecular epidemiology data of *P. aeruginosa* infection is lacking at Songklanagarind Hospital, Hat Yai, Songkhla, Thailand. Prevalence and subtypes of *P. aeruginosa* present in Songklanagarind Hospital between 2012 and 2013 were investigated using PFGE as well as correlation of subtypes with drug susceptibility. This study was intended to provide various genetic relatedness among MDR-PA and extensively drug-resistant *P. aeruginosa* (XDR-PA) strains which highly dispersed throughout Songklanagarind Hospital, especially in intensive care unit and medical ward. The data is important for planning a proper transmission control.

MATERIALS AND METHODS

Patients and bacterial strains

Bacterial samples (n = 112) including sterile fluid, such as blood, pleural fluid, cerebrospinal fluid (CSF), and non-sterile sources such as, bronchial aspirate and surgical site, from individual patients with nosocomial infection at Songklanagarind Hospital, Hat Yai, Songkhla, Thailand were collected between February 2012 to January 2013. Isolation and identification of *P. aeruginosa* were carried out by culturing on standard media and biochemical tests, as described by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2012). Demographic data (sex, age, specimen type or body site, patient's region of residence, hospitalization history, antibiogram profile and underlying disease) were retrieved from the Hospital medical records with identity redacted.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University (REC no. 55-391-04-8-3).

Antibiogram profiling

Drug susceptibility tests and interpretations were performed using a disc diffusion method according to the CLSI (2012). Each disc (Becton Dickinson, Heidelberg, Germany) contained amikacin (AK, 30 μg), ceftazidime (CAZ, 30 μg), ciprofloxacin (CIP, 5 μg), colistin (DA, 10 μg), gentamicin (GM, 10 μg), imipenem (IMP, 10 μg), meropenem (MEM, 10 μg), norfloxacin (NOR, 10 μg), Cefoperazone-Sulbactam (sulperazone)

(SPZ, 75/30 μg), and tazocin (TZP, 100 μg). MDR-PA, XDR-PA and pandrug-resistant *P. aeruginosa* (PDR-PA) isolates are defined according to the Centers for Disease Control and Prevention, USA criteria (Magiorakos *et al*, 2012). A strain resistant to at least one agent in three or more antipseudomonal antimicrobial categories is assigned MDR-PA, resistant to at least one agent in all but two or fewer antipseudomonal antimicrobial categories XDR-PA and resistant to all agents in all antipseudomonal antimicrobial categories PDR-PA.

PFGE assay

PFGE was performed as previously described by Pfaller *et al* (1992) and modified by Seifer *et al* (2005). Briefly, one $A_{600\ nm}$ amount of an overnight culture of *P. aeruginosa* in Luria Bertanii (LB) broth (Merck KGaA, Darmstadt, Germany) was incubated in 100 mM Tris pH7.2 buffer containing 100 mM EDTA, 20 mM NaCl and 0.5 mg/ml proteinase K at 55°C for 10 minutes, then an equal volume of 1.0% UltraPureTM LMP agarose (Invitrogen, Carlsbad, CA) was added and the solution placed in mold to form solid plug, which was incubated with cell lysis buffer (50 mM Tris pH 8.0, 100 mM EDTA, 0.1% SDS, 1.0% sarcosine, and 0.5 mg/ml proteinase K) at 55°C for 2 hours. Agarose plug was treated with 50 U *Xba*I for 2 hours and digested DNA was separated by 1.0% PFGE in a CHEF-DRIII system (Bio-Rad Laboratories, Hercules, CA) in 0.5X Tris-borate-EDTA buffer at 14°C and voltage of 6 V/cm for 21 hours with pulse times of 5-35 s. Lambda PFGE ladder (New England Biolabs, Beverly, MA) was used as molecular weight marker. Gel was stained with ethidium bromide and image recorded by a Gel DocTM XR^+ system (Bio-Rad Laboratories, Hercules, CA), then converted to a TIFF file.

Analysis of PFGE patterns

PFGE patterns were analyzed using Bionumerics software version 7.0 (Applied Maths, St-Martens-Latem, Belgium). Gel images were normalized by aligning bands with the size marker in each gel. Optimization and band position tolerance setting were established at 1.5%. Similarity of band patterns was calculated using a Pearson's correlation coefficient and then clustered using a dendrogram generated by an unweighted pair group of the arithmetic mean (UPGMA) method. Cluster classification of isolates was performed at >70% similarity (Lila *et al*, 2018; Huang *et al*, 2020).

Statistical analysis

Demographic data are presented as percentage (unless otherwise stated) and median value with interquartile range (IQR). All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 23 (IBM Corporation, Armonk, NY). Qualitative variables were compared using a *t*-test and Pearson Chi-square test. A p-value <0.050 is considered statistically significant.

RESULTS

Patients and *P. aeruginosa* strains

Patients (n = 112), median age of 54 years (IQR = 35-73) and 68 (61%) males at Songklanagarind Hospital were recruited during February 2012 to January 2013. Fifty-nine patients (53%) were from home and 18 (16%) were referred from other hospitals (Table 1). Half of the patients had at least one underlying disease that would predispose to *P. aeruginosa* infection, with the predominant pathologies being cardiovascular disease (cardiomyopathy and ischemic heart disease) (28%), immunocompromised status (chronic steroid use, hypothyroidism and diabetes

Table 1
Demographic profile, clinical presentation and clinical specimen of patients with *Pseudomonas aeruginosa* infection at Songklanagarind Hospital, Hat Yai, Songkhla, Thailand (February 2012 - January 2013).

Characteristic	Number (%) (n = 112)
Gender	
Male	68 (61)
Female	44 (39)
Age in years, median (IQR)	54 (35-73)
Hospitalization time in days, median (IQR)	25.5 (9.0-47.5)
Transfer from other hospitals	18 (16)
Underlying condition	
Solid organ malignancy	7 (6)
Cardiovascular disease	31 (28)
Orthopedic condition	5 (4)
Hematologic malignancy	0 (0)
Cerebrovascular disease	13 (12)
Structural lung disease	16 (14)
Immunocompromised status	21 (19)
Chronic kidney disease	8 (7)
Peripheral vascular disease	1 (1)
HIV infection	1 (1)
No underlying disease	52 (46)
Type of specimen	
Sputum	56 (50)
Peritoneal, pleural and abscess aspirates	29 (26)
Pus	15 (13)
Tissue	12 (11)
Specimen collection site	
Medical ward	42 (37)
Intensive care unit	28 (25)
Operation theater	17 (15)
Emergency room	13 (12)
Surgical ward	12 (11)
P. aeruginosa drug resistance profile	
Carbapenems	31 (28)
Cephalosporins	24 (21)
Aminoglycosides	22 (20)
Fluoroquinolones	21 (19)
Beta-lactamase inhibitors	16 (14)

IQR: interquartile range.

mellitus) (19%), and structural lung disease (pulmonary tuberculosis and chronic obstructive pulmonary disease) (14%). The median hospitalization time was 25 days (range 2-165 days, IQR 9-47). The most common clinical specimen was sputum (50%), followed by aspirates (26%); majority was collected from medical wards (37%), followed by intensive care units (25%).

Antibiogram profile

P. aeruginosa strains (n = 112) were tested against 10 different antibiotics using a disc diffusion method. The antimicrobial susceptibility of the isolates is shown in Table 2. All the isolates were sensitive to colistin 100%, and more than 75% to aminoglycosides, beta-lactamase inhibitors and carbapenems (85% to amikacin, 80% to gentamicin, 80% tazocin, 79% to ciprofloxacin, 76% to sulperazone and 75% to imipenem and meropenem). Four isolates norfloxacin-susceptible was low (57%) compared with other antimicrobials. On the other hand, the maximum resistance rate was observed to norfloxacin (43%) followed by imipenem (25%), ceftazidime and meropenem (21%), ciprofloxacin (19%), gentamicin (18%), sulperazone and tazocin (13%), and amikacin (11%), respectively. No strain was resistant to colistin. There were 18 (16%) and 5 (4%) strains classified as MDR-PA and XDR-PA respectively, with no PDR-PA strain. Overall, the majority of *P. aeruginosa* strains were sensitive to most of the antibiotics commonly used to treat infectious patients in the Hospital, with no significant difference in antibiotic sensitivity to the various antibiotic classes (Table 1).

PFGE analysis of *P. aeruginosa* strains

P. aeruginosa strains were digested with *XbaI* and separated by PFGE, generating profiles typically of 10-17 bands mainly ranging 50-500 kbp (Fig 1). Each gel-electrophoresis experiment was accompanied by *Escherichia coli* O157:H7 as standard. Comparison of the profiles were carried out using a BioNumberic software

Table 2
Antimicrobial susceptibility testing of 112 *Pseudomonas aeruginosa* isolates from patients at Songklanagarind Hospital, Hat Yai, Songkhla, Thailand (February 2012 - January 2013).

Antimicrobial agents	Intermediate resistant Number (%)	Sensitive Number (%)	Resistant Number (%)	Total
Amikacin	5 (4)	95 (85)	12 (11)	112
Ceftazidime	2 (2)	86 (77)	24 (21)	112
Ciprofloxacin	3 (3)	88 (79)	21 (19)	112
Ceftazidime	2 (2)	86 (77)	24 (21)	112
Colistin	0 (0)	69 (100)	0 (0)	69
Gentamicin	2 (2)	90 (80)	20 (18)	112
Imipenem	0 (0)	84 (75)	28 (25)	112
Meropenem	4 (4)	84 (75)	24 (21)	112
Norfloxacin	0 (0)	4 (57)	3 (43)	7
Sulperazone	12 (11)	85 (76)	15 (13)	112
Tazocin	9 (8)	89 (80)	14 (13)	112

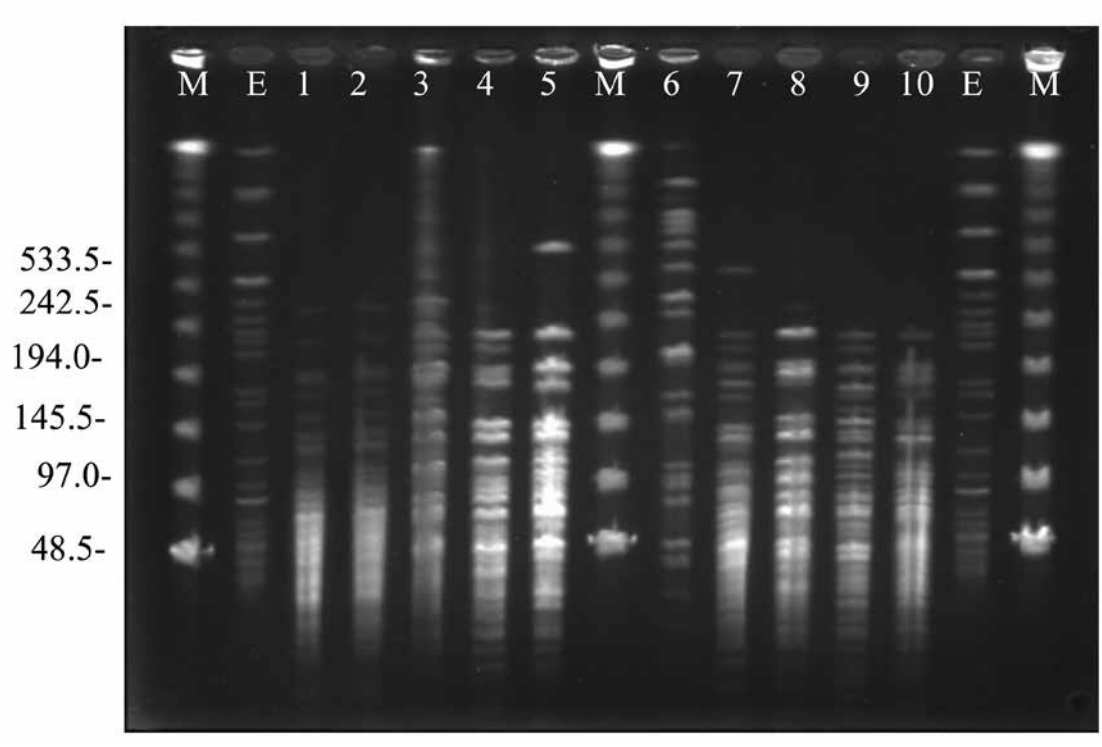

Fig 1-Representative pulsed-field gel-electrophoresis (PFGE) profiles of *Pseudomonas aeruginosa* strains from patients at Songklanagarind Hospital, Hat Yai, Songkhla, Thailand (February 2012 - January 2013).

P. aeruginosa cells were lysed *in situ* in a gel plug, digested with *XbaI*, separated by 1.0% PFGE and visualized with ethidium bromide staining. Lane M, 48.5 kbp size ladder; lane E, *Xba*I-digested *Escherichia coli* O157: H7; lanes 1-10, *P. aeruginosa* clinical strains.

(Applied Maths) and 85 (76%) strains were typeable. At a genetic relatedness (band identity) ≥70%, the strains could be clustered into seven groups (A-G) and a U group containing miscellaneous strains with <70% genetic relatedness (Fig 2). The predominant group A consisted of 33 (39%) strains, followed by group E with 27 (32%) strains; group U contained five strains. Group A and E strains were commonly isolated from patients with solid organ malignancy (19%) and from sputum (36%) (data not shown). All MDR-PA strains were present in the seven groups except in clusters F and U, while XDR-PA strains were located in groups A, E and H (Table 3). It was noticeable 40% of MDR and XDR-PA isolates were from patients in intensive care unit (ICU) and medical ward.

DISCUSSION

P. aeruginosa has become an important cause of nosocomial infections, such as urinary tract, lung and surgical site infection and sepsis (Corona-Nakamura *et al*, 2001; Fazeli *et al*, 2017) and outbreaks have been reported in ICUs, burn wound units and cancer centers (Quick *et al*, 2014; Parcell *et al*, 2018). In Thailand, spread of *P. aeruginosa* nosocomial infection has

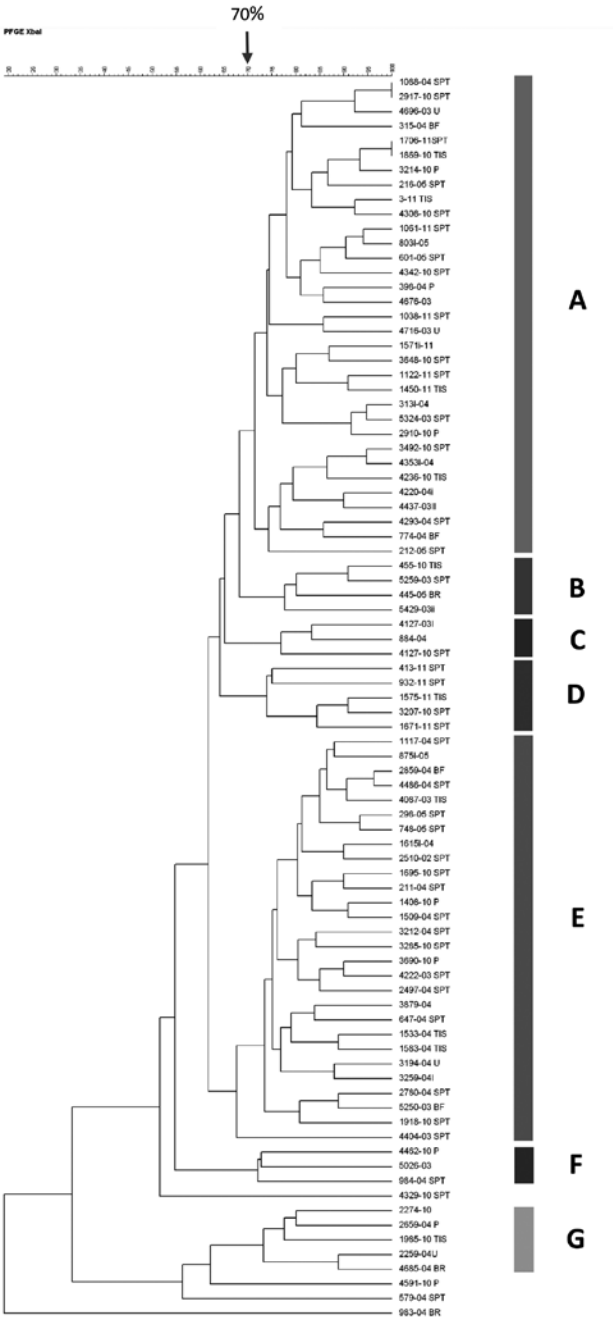

Fig 2-Dendrogram of pulsed-field gel-electrophoresis (PFGE) profiles *of Pseudomonas aeruginosa* strains from patients at Songklanagarind Hospital, Hat Yai, Songkhla, Thailand (February 2012 - January 2013).

PFGE profiles (see Fig 1) were analyzed using a Bionumerics software and similarity of band patterns was calculated using a Pearson's correlation coefficient, then a dendrogram generated employing an unweighted pair group of the arithmetic mean method. Clustering into groups of isolates was performed at >70% similarity. Scale denotes percent similarity.

Table 3
Pulsed-field gel-electrophoresis (PFGE) group, source, hospital collection location, antibiogram profile, and isolation date of multidrug-resistant (MDR-PA) and extensively drug-resistant (XDR-PA) *Pseudomonas aeruginosa* strains from patients at Songklanagarind Hospital, Hat Yai, Songkhla, Thailand.

PFGE group	Resistant category	Source	Hospital ward	Isolation code	AK	CAZ	GM	IMP	MEM	NOR	CIP	SPZ	TZP	DA	Collection date (day/month/year)
A	MDR	TIS	OPD	1450-11 TIS PS	I	R	S	S	R	NA	R	R	R	S	08/11/2012
A	MDR	SPT	Medical ward	3492-10 SPT PS	S	R	S	R	R	NA	S	R	R	NA	18/10/2012
A	MDR	Pus	Medical ward	398-04 P PS	R	R	R	R	NA	NA	R	S	S	S	03/04/2012
A	MDR	SPT	ICU	4293-04 SPT PS	R	R	R	R	NA	NA	S	S	S	S	28/04/2012
A	MDR	HC	Medical ward	4353I-04	R	S	R	R	NA	NA	R	S	S	S	28/04/2012
A	MDR	BF	Medical ward	4676-03 PS	S	R	R	R	R	NA	S	I	S	S	27/03/2012
A	MDR	SPT	Medical ward	601-05 SPT PS	S	R	R	R	I	NA	R	I	I	S	04/05/2013
B	MDR	TIS	OPD	455-10 TIS PS	R	R	R	S	S	NA	I	I	I	NA	03/10/2012
D	MDR	SPT	Medical ward	1671-11 SPT PS	S	R	R	R	R	NA	R	R	R	S	10/11/2012
C	MDR	HC	Medical ward	884-04 PS	I	R	R	R	NA	NA	R	R	R	NA	05/04/2012
E	MDR	SPT	Medical ward	1918-10 SPT PS	I	R	R	R	R	NA	R	I	I	S	10/10/2012
E	MDR	PTBD (BF)	Surgical ward	2859-04 BF	S	NA	S	R	R	NA	R	R	R	S	19/04/2012
E	MDR	SPT	ICU	3285-10 SPT PS	I	S	R	S	R	NA	R	R	R	NA	23/01/2012
G	MDR	BF (JP)	Medical ward	2259-04U PS	I	S	S	R	R	R	R	NA	R	S	03/04/2012

Table 3 (Continued)

PFGE group	Resistant category	Source	Hospital ward	Isolation code	AK	CAZ	GM	IMP	MEM	NOR	CIP	SPZ	TZP	DA	Collection date (day/month/year)
E	XDR	SPT	ICU	298-05SPT PS	R	R	R	R	R	NA	R	R	R	S	02/05/2012
E	XDR	SPT	ICU	647-04 SPT PS	R	R	R	R	R	NA	R	R	R	S	04/04/2012
E	XDR	SPT	ICU	748-05 SPT PS	R	R	R	R	R	NA	R	R	R	NA	04/05/2012
A	XDR	BF (hip)	OPD	315-04 BF PS	R	R	R	R	R	NA	R	R	R	S	02/04/2012
U	XDR	Ascites	ICU	983-04 BF PS	R	R	R	R	R	NA	R	R	R	S	06/04/2012

BF: body fluid; BF (JP): body fluid Jackson-Pratt drain; HC: hemoculture; ICU: intensive care unit; OPD: out-patient department; PTBD (BF): percutaneous transhepatic biliary drainage (body fluid); SPT: sputum; TIS: tissue, AK: amikacin, 30 μg; CAZ: ceftazidime, 30 μg; CIP: ciprofloxacin, 5 μg; DA: colistin, 10 μg; GM: gentamicin, 10 μg; IMP: imipenem, 10 μg; MEM: meropenem, 10 μg; NOR: norfloxacin, 10 μg; SPZ: sulperazone, 75/30 μg; TZP: tazocin, 100 μg; I: intermediate resistant; NA: not applicable; R: resistant; S: sensitive.

been reported in 85 hospitals during 2000-2018 (NARST, 2018). In the lower southern regions of Thailand, *P. aeruginosa* infection rate is 13.5% in eight hospitals (NARST, 2019).

At Songklanagarind Hospital from February 2012 to January 2013, almost half of *P. aeruginosa*-infected patients had at least one underlying disease (mainly cardiovascular disease). *P. aeruginosa* strains were still equally sensitive to the four major classes of antimicrobials prescribed at the Hospital, but, worryingly, a quarter of the strains could be classified as MDR- and XDR-PA, but none as PDR-PA. The prevalence of MDR and XDR pathogens can vary among wards, hospitals and regions depending on antibiogram profiles in each circumstances and policy governing antibiotics disbursement (Cholley *et al*, 2010; Bayani *et al*, 2013; Sabir *et al*, 2013; Biswal *et al*, 2014; Chittawatanarat *et al*, 2014). Amikacin was the most effective antibiotic (11% resistant) and ceftazidime the least effective (21% resistant), in concordance with the findings of Chander and Raza, 2013 conducted in Kanthmandu, Nepal. Percent resistance to ceftazidime and imipenem was lower than that (21% and 25% respectively) previously reported (Thompat and Sudjaroen, 2009). This phenomenon might be due to improvement in management and maintenance of infection control procedures (Wang *et al*, 2012, Matt *et al*, 2014), re-enforcing the prudent use of antibiotics and strict adherence to guidelines for their empirical treatment. Recently, cephalosporin, ciprofloxacin and carbapenem were reported to be productive antimicrobial agents against XDR-PA bacteremia in the ICU at Roi-Et Hospital, Thailand (Woradet *et al*, 2020), whereas amikacin or gentamicin were

effectively used for MDR-PA infection (Katvoravutthichai *et al*, 2016).

Despite a variety of methods for molecular typing of microbes, *viz* Diversilab repetitive-sequence-based PCR, multilocus sequencing typing, multiple locus variable-number tandem repeat analysis, and next generation sequencing technology), each technique has its own different advantages and disadvantages (Chen *et al*, 2018). PFGE, an older, more time consuming and labor-intensive method, is still regarded as an accurate and reproducible tool for epidemiology investigations in a hospital setting (Parizad *et al*, 2016). However, in this study, there were 27 isolates (24.1%) that were non-typeable by PFGE. Strains that could not be typed might be due to degraded DNA, as observed with genetic typing of *Clostridium difficile*, *Klebsiella* spp, *Ralstonia* spp, and *Vibrio parahaemolyticus* (Römling and Tümmler, 2000; Meletis and Bagkeri 2013).

Of the genetically typeable *P. aeruginosa* clinical strains (n = 85), PFGE profiles revealed all but seven strains with ≥70% genetic relatedness, suggesting that the latter seven strains were probably hospital acquired. The evidence that genetically related *P. aeruginosa* strains were clustered into several distinguishable groups would imply that these strains have been in circulation in Songklanagarind Hospital for some period of time prior to the present survey. Colonization seems to play a major role in the spread of *P. aeruginosa* within ICUs, as noted by Parcell *et al* (2018). In addition, MDR-PA and XDR-PA have been mostly discovered in sputum and spread in medical ward and ICU, respectively. The XDR-PA infections among ICU patients were also found in Roi Et Hospital (Woradet *et al*, 2019).

In conclusion, the study confirms the usefulness of pulsed-field gel-electrophoresis in molecular epidemiology investigations of *Pseudomonas aeruginosa* strains circulating in a hospital. Primary screening using molecular techniques for identification of predominant clones should be of assistance in the control of colonization and transmission within a tertiary care center. However, there has not been any study of community-acquired *P. aeruginosa* infection or of infection in healthcare workers, and further investigations in these areas are warranted.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Medicine, Prince of Songkla University, (grant no. 55-391-04-8-3.).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Bayani M, Siadati S, Rajabnia R, Taher AA. Drug resistance of *Pseudomonas aeruginosa* and *Enterobacter cloacae* isolated from ICU, Babol, Northern Iran. *Int J Mo. Med* 2013; 2: 204-9.

Biswal I, Arora BS, Kasana D, Neetushree. Incidence of multidrug resistant *Pseudomonas aeruginosa* isolated from burn patients and environment of teaching institution. *J Clin Diagn Res* 2014; 8: DC26-9.

Chander A, Raza MS. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* clinical isolates at a tertiary care hospital in Kathmandu, Nepal. *Asian J Pharm Clin Res* 2013; 6: 235-8.

Chen JW, Lau YY, Krishnan T, Chan KG, Chang CY. Recent advances in molecular diagnosis of *Pseudomonas aeruginosa*

infection by state-of-the-art genotyping techniques. *Front Microbiol* 2018; 9: 1104.

Chittawatanarat K, Jaipakdee W, Chotirosniramit N, Chandacham K, Jirapongcharoenlap T. Microbiology, resistance patterns, and risk factors of mortality in ventilator-associated bacterial pneumonia in a northern Thai tertiary-care university based general surgical intensive care unit. *Infect Drug Resist* 2014; 7: 203-10.

Cholley P, Gbaguidi-Haore H, Bertrand X, *et al*. Molecular epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in a French university hospital. *J Hosp Infect* 2010; 76: 316-9.

Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing; Twenty-second Informational Supplement* M100-S22, Wayne, PA: CLSI; 2012.

Corona-Nakamura AL, Miranda-Novales MG, Leanos-Miranda B, *et al*. Epidemiologic study of *Pseudomonas aeruginosa* in critical patients and reservoirs. *Arch Med Res* 2001; 32: 238-42.

Fazeli H, Nasr Esfahani B, Sattarzadeh M, Mohammadi Barzelighi H. Antibiotyping and genotyping of *Pseudomonas aeruginosa* strains isolated from Mottahari Hospital in Tehran, Iran by ERIC-PCR. *Infect Epidemiol Microbiol* 2017; 3: 41-5.

Grundmann H, Schneider C, Hartung D, Daschner FD, Pitt TL. Discriminatory power of three DNA-based typing techniques for *Pseudomonas aeruginosa*. *J Clin Microbiol* 1995; 33: 528-34.

Hu H, Manos J. Pulsed-field gel electrophoresis of *Pseudomonas aeruginosa*. *Methods Mol Biol* 2015, 1301: 157-70.

Huang J, Lan F, Lu Y, Li B. Characterization of integrons and antimicrobial resistance in *Escherichia coli* sequence Type 131 isolates. *Can J Infect Dis Med Microbiol* 2020; 2020: 3826186.

Katvoravutthichai C, Boonbumrung K, Tiyawisutsri R. Prevalence of β-lactamase classes A, C, and D among clinical isolates of *Pseudomonas aeruginosa* from a tertiary-level hospital in Bangkok, Thailand. *Genet Mol Res* 2016; 15: 10.4238/gmr.15038706.

Lila G, Mulliqi G, Raka L, Kurti A, Bajrami R, Azizi E. Molecular epidemiology of *Pseudomonas aeruginosa* in University Clinical Center of Kosovo. *Infect Drug Resist* 2018; 11: 2039-46.

Maatallah M, Bakhrouf A, Habeeb MA, *et al*. Four genotyping schemes for phylogenetic analysis of *Pseudomonas aeruginosa*: comparison of their congruence with multi-locus sequence typing. *PLoS One* 2013; 8: e82069.

Magiorakos AP, Srinivasan A, Carey RB, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268-81.

Matt B, Mitteregger D, Renner S, Presterl E, Assadian O, Diab-Elschahawi M. Successful implementation of infection control strategies prevents *P. aeruginosa* transmission among cystic fibrosis patients inside the hospital. *GMS Hyg Infect Control* 2014; 9: Doc20.

Meletis G, Bagkeri M. *Pseudomonas aeruginosa*: multi-drug-resistance development and treatment options, 2013 [cited 2013 May 29]. Available from: URL: https://www.intechopen.com/books/infection-control/pseudomonas-aeruginosa-multi-drug-resistance-development-and-treatment-options

National Antimicrobial Resistance Surveillance Thailand (NARST). Situation of antimicrobial resistance during 2000-2018, 2018 [cited 2018 May 18]. Available from URL: http://narst.dmsc.moph.go.th/data/AMR%202000-2018-12M.pdf

National Antimicrobial Resistance Surveillance Thailand (NARST). Percentage of non-susceptible organisms in Thailand, January - June 2018, 2019 [cited 2019 Jul 02]. Available from URL: http://narst.

dmsc.moph.go.th/data/map2561-12m.pdf

Pagani L, Mantengoli E, Migliavacca R, *et al*. Multifocal detection of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum beta-lactamase in Northern Italy. *J Clin Microbiol* 2004; 42: 2523-9.

Parcell BJ, Oravcova K, Pinheiro M, *et al*. *Pseudomonas aeruginosa* intensive care unit outbreak: winnowing of transmissions with molecular and genomic typing. *J Hosp Infect* 2018; 98: 282-8.

Parizad EG, Parizad EG, Valizadeh A. The application of pulsed field gel electrophoresis in clinical studies. *J Clin Diagn Res* 2016; 10: DE01-4.

Pfaller M, Hollis R, Sader H. Chromosomal restriction fragment analysis by pulsed-field gel electrophoresis. In: Isenberg HD, editor. Clinical microbiology procedures handbook. Suppl 1. Washington DC: ASM Press; 1994. p. 10.5.c.1.-12.

Quick J, Cumley N, Wearn CM, *et al*. Seeking the source of *Pseudomonas aeruginosa* infections in a recently opened hospital: an observational study using whole-genome sequencing. *BMJ Open* 2014; 4: e006278.

Römling U, Tümmler B. Achieving 100% typeability of *Pseudomonas aeruginosa* by pulsed-field gel electrophoresis. *J Clin Microbiol* 2000; 38: 464-5.

Sabir R, Danish Alvi SF, Fawwad A. Antimicrobial susceptibility pattern of aerobic microbial isolates in a clinical laboratory in Karachi-Pakistan. *Pak J Med Sci* 2013; 29: 851-5.

Sader HS, Huband MD, Castanheira M, Flamm RK. *Pseudomonas aeruginosa* antimicrobial susceptibility results from four years (2012 to 2015) of the International Network for Optimal Resistance Monitoring Program in the United States. *Antimicrob Agents Chemother* 2017; 61: e02252-16.

Santos-Sanches I, Chambel L, Tenreiro R. Pulsed-field gel electrophoresis (PFGE): application in population structure studies of bovine mastitis-causing streptococci. *Methods Mol Biol* 2015; 1247: 323-34.

Seifert H, Dolzani L, Bressan R, *et al*. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; 43: 4328-35.

Spiers AJ, Buckling A, Rainey PB. The causes of *Pseudomonas* diversity. *Microbiology* 2000; 146: 2345-50.

Thompat W, Sudjaroen Y. Characterization and antibiotic susceptibility profile of nosocomial pathogens isolated from cancer patients. *Thai Cancer J* 2009; 29:176-83.

Wang LJ, Sun Y, Song WL, Zhang ZJ, Liu CF. Changes of drug-resistance of *Pseudomonas aeruginosa* in pediatric intensive care unit. *Zhonghua Er Ke Za Zhi* 2012; 50: 657-63. [in Chinese]

Woradet S, Chaimay B, Putthanachot N, Sangsa N, Sirisa P. Antimicrobial agents administration among patients with extensively drug-resistant *Pseudomonas aeruginosa* infection in intensive care unit in tertiary care: a hospital-based study. *J Clin Diagn Res* 2020; 14: 1-4.

Woradet S, Putthanachot N, Chaimay B, Sangsa N, Sirisa P. Determination of factors associated with extensively drug-resistant *Pseudomonas aeruginosa* infection among intensive care unit patients at Roi Et Hospital, Thailand. *Southeast Asian J Trop Med Public Health* 2019; 50: 266-74.