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Original Article

Inhibition of the clinical isolates of *Acinetobacter baumannii* by *Pseudomonas aeruginosa: In vitro* assessment of a case-based study

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KEYWORDS
Pseudomonas aeruginosa;
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Abstract *Background:* The global rise in nosocomial infections associated with gram-negative bacteria and the spread of multi-drug resistant *Acinetobacter baumannii* (MDR-AB) pose public health concerns. This study investigates the inhibitory effects and possible inhibitory mechanism of *Pseudomonas aeruginosa* (PA) on selected clinical strains of *A. baumannii* (AB) isolated from Taiwanese patients.

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baumannii;
Methicillin-resistant *Staphylococcus aureus* (MRSA);
Phenazine-1-carboxamide (PCN)

Methods: Four and eight clinical strains of AB and PA, respectively, were randomly selected from the bacterial collection of Feng-Yuan Hospital, Taiwan. Antimicrobial-susceptibility was performed on the AB strains. Inhibition potential of the PA strains against AB was assessed by measuring the inhibition zones. In vitro analysis using phenazine-1-carboxamide (PCN) was conducted to assess the possible inhibitory mechanism of PA, which was later confirmed in the clinical isolates by liquid chromatography-mass spectrometry.
Results: All the clinical AB strains showed resistance to the eleven antibiotics and were classified as MDR-AB. The nine PA strains exert either a high (PA3596, PA3681, PA3772, and ATCC27853) or a low (PA3613, PA3625, PA3712, PA3715, and PA3744) degree of inhibition against AB strains. 0.25 mg/ml PCN had a clearer inhibition zone than 0.05 mg/ml PCN, suggesting a dose-dependent inhibition of PCN on the AB strains. The four PA strains that demonstrated a high degree of inhibition had a relatively high amount of PCN.
Conclusion: Selected strains of PA exert inhibitory actions on MDR-AB with PCN being a possible inhibitory agent. This finding raises the possibility of developing effective therapeutic antibiotics and disinfectant from specific components of PA for the treatment and control of *Acinetobacter*-associated infections in hospital settings.
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Introduction

There has been a global increase in nosocomial infections that are caused by gram-negative bacteria such as *Acinetobacter baumannii* (AB),[1] with attendant human infections such as pneumonia, septicemia, and urinary tract infections.[2] In Taiwan, there is a high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA),[3] penicillin-resistant *Streptococcus pneumoniae*,[4] extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*,[5] and extensively or multidrug-resistant (XDR or MDR) AB.[6,7] The spread of MDR *Acinetobacter* strains among critically ill and hospitalized patients raises public health concerns,[8] which is further complicated by the availability of only a few options of clinically effective treatment.[9] In addition, infections due to MDR-AB and other *Acinetobacter* species cause high in-hospital-associated (21.8%) and overall (26%–60%) mortality rates,[10,11] and may potentially cause community-acquired infections.[12,13] These observations underscore the pressing need to develop viable antimicrobial interventions.

At the Respiratory Care Ward of Feng-Yuan Hospital, Taiwan, we recovered MDR-AB from a sputum culture of a female patient with chronic respiratory failure. At the time, the patient did not have any clinical symptoms other than chronic respiratory failure and chest X-ray radiography showed no sign of pneumonia foci. Due to this reason, no antibiotic was administered. Some weeks later, however, the patient showed some clinical symptoms of pneumonia. *Pseudomonas aeruginosa* (PA) was recovered from her sputum sample, but surprisingly, MDR-AB was not, suggesting that PA may inhibit the growth of MDR-AB *in vivo*. Previously, PA strains have been shown to produce a variety of compounds, including phenazine-1-carboxamide (PCN), phenazine-1-carboxylic acid, pyocyanin and other small molecules, to inhibit the growth of MRSA, vancomycin intermediate-resistant *S. aureus* (VISA), *Corynebacterium* spp. and *Moraxella catarrhalis* strains.[14–16] Therefore, this study sought to evaluate the inhibitory capacity of the clinically isolated PA against MDR-AB, and PCN was identified as the inhibitory compound. We also elucidated the possible inhibitory mechanism, particularly that of PCN.

Methods

Sample collection & characterization of bacterial strains

Four and eight clinical strains of AB and PA, respectively, were randomly selected from the de-identified bacterial collection of Feng-Yuan Hospital, Department of Health, Taichung, Taiwan. These 12 strains were independently isolated from otherwise aseptic samples of the respiratory tract, bloodstream, and urinary tract of patients. All the strains were stored in tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD) containing 25% (v/v) glycerol at −20 °C. The strains were initially identified by the VITEK®-2 automated microbial identification system (BioMérieux, Inc. Durham, NC), and later confirmed by the BD Phoenix™ automated identification and susceptibility-testing system (Becton, Dickinson and Company, Franklin Lakes, New Jersey). In addition, one ATCC AB strain (ATCC17978) and one ATCC PA strain (ATCC 27853) purchased from American Type Culture Collection (Manassas, VA) were used as controls.

Antimicrobial-susceptibility test

Antimicrobial-susceptibility test of the five AB strains was performed by the BD Phoenix™ automated identification and susceptibility-testing system using the Clinical and Laboratory Standards Institute (CLSI) reference guidelines (NCCLS, 2004).[17]

Pulsed-field gel electrophoresis

In-gel digestion of the chromosomal DNA of the nine PA strains by *Spe*I was performed and the digested fragments were separated by pulsed-field gel electrophoresis (PFGE) according to the method of Chen et al.[18] The PFGE patterns produced were initially analyzed by visual inspection of the photographs of the strained gels. Later, genetic similarities between pairs of patterns were calculated by Nei and Li's F statistic.[19] A matrix of the F values for all pairs of patterns was prepared and used to construct a dendrogram by the NTSYS-PC software (Numerical Taxonomy and Multivariate Analysis System, version 7.50) from Applied Biostatistics, Inc (Setauket, NY).

Inhibitory spectra

The modified agar disk diffusion method of Xu et al.[16] was employed to test the inhibition of the nine PA strains against the five AB strains. The experimental procedures are shown in the flowchart in Fig. 1. For the PA strains, the overnight cultures were inoculated into TSB broth and incubated at 37 °C till $OD_{600} = 0.6$. The bacterial cultures were centrifuged, and the supernatant was filtered through a 0.22 μm filter (Pall Corporation, Ann Arbor, MI) to remove residual bacteria. Five microliter of the cell-free cultural supernatant was then spotted onto the soft agar plates, where AB strains were seeded. This set of plates was named the "sup filter plates". The gridded PA plates that were stored at 4 °C (on the third day) were warmed up at room temperature and used to pick some bacteria onto the AB soft agar plates and were named the "colony plates". The sup filter and colony plates were incubated at 37 °C for 45 h. During the 45-hr incubation period, the plates were observed for the appearance of clear zones at 16 h, 22 h, and 45 h. Photographs were taken and the diameters of the inhibition zone in cm were measured and recorded.

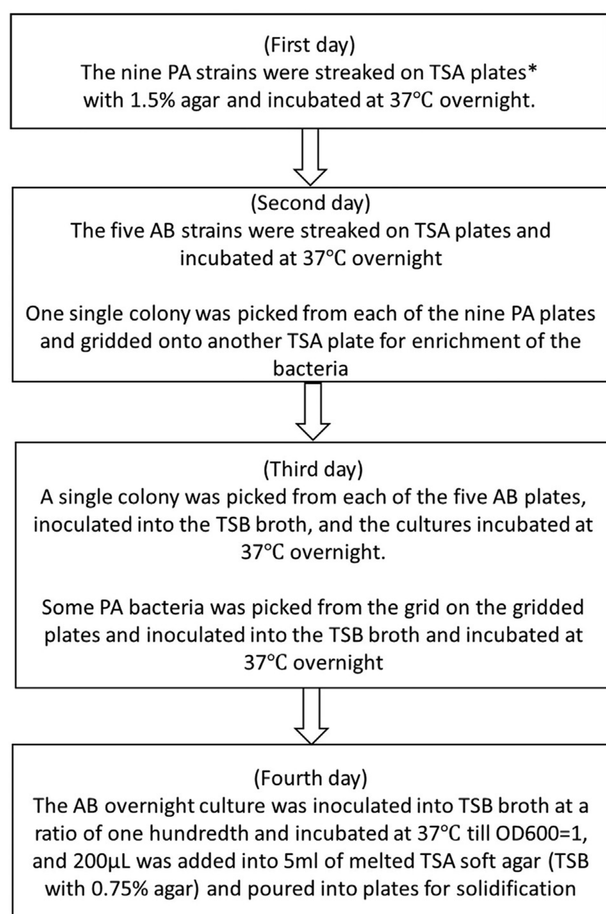

Fig. 1. Methodology of inhibitory spectra.

The inhibitory effect of phenazine-1-carboxamide

Phenazine-1-carboxamide (PCN) (CAS 550-89-0) was purchased from Toronto Research Chemicals, Toronto, Canada, and a 0.25 mg/ml solution in 5% acetonitrile was prepared. This solution was further diluted with distilled water into 0.05 mg/ml. Five microliter of both solutions was spotted onto the AB soft agar plates, and 5% acetonitrile and 1% acetonitrile were also applied as negative controls. The plates were incubated at 37 °C for 16 h and the appearances of inhibition zones were checked at 4 h and 16 h.

Liquid chromatography-mass spectrometric (LC/MS) analysis

The 16-h culture of the nine PA strains was centrifuged and the supernatants were filtered through a 0.22 μm filter. One hundred microliter of the filtrate was added to 400 μL extraction solvent (methanol), vortex-mixed, followed by ultrasonication-assisted extraction for one minute. The solution was incubated at −20 °C for 20 min. The solution was then centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatant was filtered through a 0.22 μm filter and then vacuum-dried. The dried sample was re-dissolved in 100 μL water/acetonitrile (95:5, v/v), vortexed-mixed, followed by ultrasonication-assisted extraction for one minute. The solution was centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was transferred to vials for LC/MS analysis. The samples were assessed using a Waters Xevo TQ tandem quadrupole mass spectrometer (Micromass MS Technologies, Manchester, UK) with Waters Acquity ultra-performance LC system (Waters Corp., Milford, MA, USA). One microliter of the sample was chromatographed on a Waters Acquity Ultra-performance LC Atlantis T3 Column (100 Å, 3 μm, 2.1 mm × 100 mm) (Waters Corp., Milford, MA, USA). Mobile phase A and B were 0.1% formic acid in ddH_2O and 0.1% formic acid in ACN, respectively. The flow rate was set at 250 μL/min. The gradient began with 5% (0 → 2 min), 50% (2.1 → 4 min), 80% (4.1 → 6 min), 90% (6.1 → 8 min), 90% (8 → 10 min), and 5% (10 → 15 min). The temperature of column and the auto-sampler were maintained at 35 °C and 16 °C, respectively. LC/MS was operated in positive ion mode by applying a capillary voltage of 3 kV. The temperature of the heated capillary and desolvation in the electrospray ionization source was set at 150 °C and 350 °C, respectively. The desolvation gas

flow was set at 600 L/min. The collision gas flow was set at 0.24 ml/min. Multiple reaction monitoring mode was used for data acquisition, and the transition and MS parameters of PCN are shown below .[20]

Compound name	Retention time (min)	Ion mode	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell time (s)	Collision energy (V)	Cone voltage (V)
phenazine-1-carboxamide	6.5	Positive	224.75	207.03[a]	0.025	12	38
				152.25		38	
				102.01		46	
				125.21		46	
				128.20		38	

[a] Quantifying ion.

Results

Antimicrobial-susceptibility test

The results of the antibiotic susceptibility of the five AB strains are presented in Table 1. The four clinical test samples (AB3652, AB3668, AB 36589, and AB3748) exhibited resistance to all of the eleven antibiotics tested, they were therefore defined as MDR-AB. However, the control ATCC17978 was susceptible to ten out of the eleven tested antibiotics; hence, it was defined as non-MDR-AB.

Pulsed-field gel electrophoresis (PFGE)

For genotyping the nine PA strains, the bacterial genome was digested with *Spe*I and analyzed by PFGE (*Spe*I-PFGE) with *Xba*I-digested *Salmonella braenderup* H9812 as the molecular weight marker, as described by Chen et al.[18] As shown in Fig. 2A, the *Spe*I-PFGE pattern of PA3715 and PA3772 revealed a 94.7% similarity in the PFGE patterns (Fig. 2B). These two strains were further digested with *XbaI* and the *XbaI*-PFGE patterns were almost the same (Fig. 2A), suggesting that these two strains were isolated from the same origin. The *Spe*I-PFGE patterns of the other PA strains showed a similarity that was less than 73%, suggesting that they were of independent origins.

Inhibitory spectra

Inhibition of the colony and the cell-free cultural supernatant of the nine PA strains against the five AB strains were tested. Fig. 3A and Fig. 3B show representative photographs of the inhibition zone on the colony (colony plates) and cell-free cultural supernatant (sup filter plates), respectively. For every PA strain that was cultured against each AB strain, the inhibition was tested in triplicate and examined after 16-, 22-, and 45-hr incubation, and the results are summarized in Supplementary tables 1–4. For each of the AB strain, it appears that the diameter of the inhibition zones of the colony plates increases as the incubation time increases, but not for those of the sup filter plates. An assessment of the results of the sup filter plates showed that many of the nine PA strains showed inhibition zones against AB3652, AB3659, AB3768, and ATCC17978, but none showed inhibition zones against AB3748 during the 16, 22, and 45-h incubation period. AB3748 was less sensitive than the other four AB strains to the inhibitory material secreted by the nine PA strains.

Table 2 presents the results of the nine PA strains against the five AB strains after 16 h of incubation. To roughly quantitate the inhibition activity of each PA strain, we defined the inhibition ability as arbitrary units (AUs) by counting the times that inhibition zones appeared either from the colony plate or sup filter plate. The inhibition abilities of the nine PA strains against the five AB strains after 16 h incubation are listed in the rightmost column in Table 2 suggesting that the nine PA strains may be divided into two groups: the high activity group (PA3596, PA3681, PA3772, and ATCC27853) and the low activity group (PA3613, PA3625, PA3712, PA3715, and PA3744).

Table 1 Antibiotic susceptibility test of the five *A. baumannii* strains as represented by the Antimicrobial minimum inhibitory concentration (MIC; μg/ml).

Strain	SAM[a]	GM	AN	CIP	LVX	CAZ	FEP	IPM	MEM	TZP	SXT
AB3652	>16/8, R[b]	>8, R	>32, R	>2, R	>4, R	>16, R	>16, R	>4, R	>4, R	>64/4, R	>2/38, R
AB3668	16/8, I	>8, R	>32, R	>2, R	>4, R	>16, R	>16, R	>4, R	>4, R	>64/4, R	>2/38, R
AB3659	>16/8, R	>8, R	>32, R	>2, R	>4, R	>16, R	>16, R	>4, R	>4, R	>64/4, R	>2/38, R
AB3748	>16/8, R	>8, R	>32, R	>2, R	>4, R	>16, R	>16, R	>4, R	>4, R	>64/4, R	>2/38, R
ATCC17978	≤4/8, S	4, S	≤8, S	≤0.5, S	≤1, S	4, S	4, S	≤0.25, S	≤0.25, S	8/4, S	R > 2/38, R

[a] SAM = ampicillin/sulbactam, GM = gentamicin, AN = amikacin, CIP = ciprofloxacin, LVZ = levofloxacin, CAZ = ceftazidime, FEP = cefepime, IPM = imipenem, MEM = meropenem, TZP = piperacillin/tazobactam, SXT = trimethoprim/sulfamethoxazole.
[b] The letters R, I and S of each drug are defined according to the CLSI guideline.

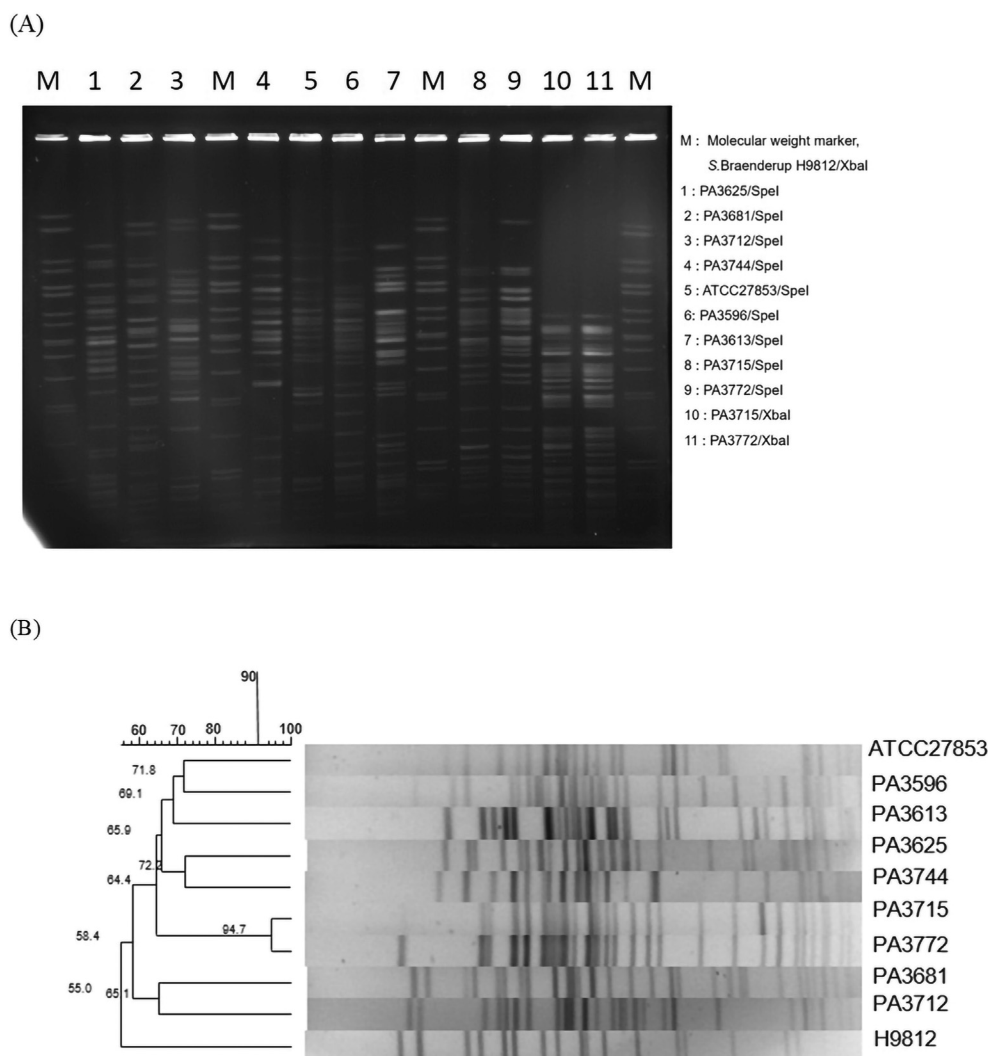

Fig. 2. (A) Pulsed-field gel electrophoresis (PFGE) patterns of the nine PA strains (B) Similarity analysis of the *Spe*I-PFGE patterns of the nine PA strains. The *Xba*I-PFGE pattern of *S. braenderup* H9812 is used as out group control.

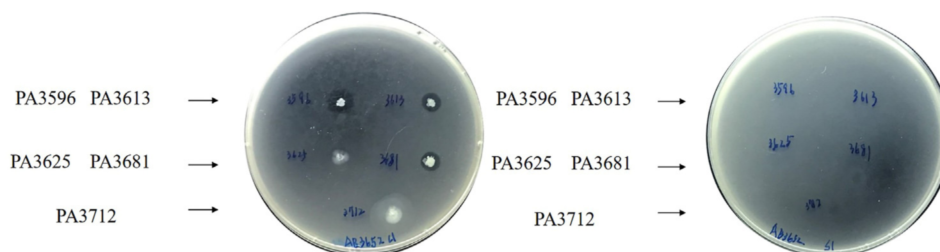

Fig. 3. Representative photographs of the colony plates (A) and sup filter plates (B). (A) In this representative colony plate, PA3596, PA3613, and PA3681 had inhibition zones against AB3652 with a diameter of 0.9, 0.7 and 0.7 cm, respectively, whereas PA3625 and PA3712 did not show any inhibition zone; (B) In this representative sup filter plate, PA3681 and PA3712 had inhibition zones against AB3652 with diameter of both 0.4 cm PA3596, PA3613 and PA3625 did not show any inhibition zone.

Inhibitory effect of phenazine-1-carboxamide (PCN)

Previously Cardozo et al. demonstrated that PCN in the cultural supernatant of one PA strain had inhibitory activity against MRSA strains.[15] In this study, commercial PCN was first used to check whether it inhibits the growth of the five AB strains. Both 0.25 mg/ml and 0.05 mg/ml PCN solution showed inhibition zones against all five AB strains tested after 4 h and 16 h of incubation (Fig. 4). Furthermore, for each AB strain, 0.25 mg/ml PCN had a clearer inhibition zone than the 0.05 mg/ml PCN had, suggesting a

Table 2 Summary of the nine *P. aeruginosa* strains against the five *A. baumannii* strains after 16-h incubation. C and FS denote colony and filtered supernatant, respectively.

P. aeruginosa	*A. baumannii*										Inhibition activity (AU)
	AB3652		AB3659		AB3748		AB3768		ATCC17978		
	C	FS	C	FS	C	FS	C	FS	C	FS	
PA3596	–/–/-	–/–/-	0.4/0.4/0.4	0.8/0.6/0.8	0.4/0.4/-	–/–/-	–/–/-	–/–/-	–/–/-	0.6/0.5/0.7	11
PA3613	–/–/-	–/–/-	–/–/-	0.4/0.5/0.7	–/–/-	–/–/-	–/–/-	–/–/-	–/–/-	-/0.2/0.4	5
PA3625	–/–/-	-/0.4/0.4	–/–/-	–/–/-	–/–/-	–/–/-	–/–/-	–/–/0.4	–/–/-	0.3/0.2/-	5
PA3681	0.6/-/0.6	0.4/0.3/-	–/–/-	0.2/0.2/0.3	-/0.4/-	–/–/-/	–/–/-	0.4/0.3/0.4	0.4/0.4/0.4	0.3/0.3/0.3	17
PA3712	–/–/-	03/–/–	–/–/-	-/0.2/-	–/–/-	–/–/-	–/–/-	0.3/–/–	–/–/-	0.7/0.5/-	5
PA3715	–/–/-	–/–/0.4	–/–/-	0.3/0.3/0.4	–/–/-	–/–/-	–/–/-	–/–/0.4	–/–/-	0.4/0.4/0.3	8
PA3744	-/0.4/-	–/–/-	–/–/-	–/–/-	0.3/-/0.2	–/–/-	0.6/0.6/-	–/–/-	–/–/-	–/–/-	5
PA3772	0.4/0.6/0.6	0.4/0.3/0.4	–/–/-	0.5/0.6/0.6	0.4/0.4/0.4	–/–/-	0.5/0.6/0.8	0.4/0.3/0.4	–/–/-	0.6/0.5/0.5	21
ATCC27853	–/–/-	0.5/0.4/0.4	0.3/–/–	0.2/0.3/-	–/–/-	–/–/-	0.5/0.5/0.5	0.5/0.4/0.4	–/–/-	–/–/-	12

[a]The numbers represent the diameter of the inhibition zone in cm of the three replicates, that are separated by the two slashes, whereas "-" represents no inhibition zone.

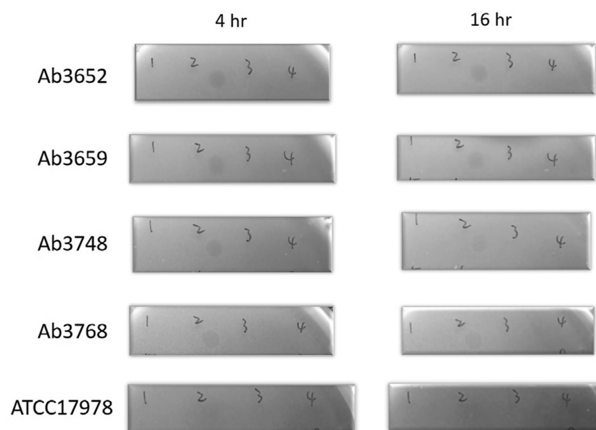

Fig. 4. Inhibition spectra of PCN against AB strains. Five microliters of four solutions were spotted onto plates seeded with different AB strains as indicated. Photographs were taken after 4- and 16-hr incubation. 1, 5% acetonitrile; 2, 0.25 mg/ml PCN in 5% acetonitrile; 3, 1% acetonitrile; 4, 0.05 mg/ml PCN in 1% acetonitrile.

dose-dependent inhibition effect of PCN on the five AB strains.

LC/MS analysis of PCN in the supernatant of the nine PA strains

The relative quantification of PCN concentration in the cell-free culture medium of the nine PA strains was measured by LC/MS (Fig. 5). Among the nine PA strains, four (PA3596, PA3681, PA3772, and ATCC27853) had a relatively high amount of PCN, whereas the other five PA strains (PA3613, PA3625, PA3715, PA3712, and PA3744) had a relatively low or no detectable amount of PCN in their cell-free culture medium. Table 2 shows that the former four strains belong to the high activity group (with inhibition activities of 11, 17, 21, and 12 AU, respectively), whereas the latter five strains belong to the low activity group (with inhibition activity of 5, 5, 5, 8, and 5 AU, respectively), indicating the antimicrobial activity against AB strains correlates well with the extracellular quantity of PCN produced by the PA strains.

Discussion

A. baumannii is one of the six most important nosocomial microbes with multidrug-resistant capacity worldwide. Its mortality rate has been estimated to be 35%.[21] Although effective infection control measures may help in decreasing the incidence of *A. baumannii* infections,[22] the availability of very few effective antibiotics for treating associated infections emphasizes the pressing need to develop alternative and viable therapy. Based on the case presented at Feng-Yuan Hospital, some clinical isolates of PA may inhibit multidrug-resistant strains of AB, suggesting that the mechanism of the bactericidal properties of different strains of PA necessitates further characterization and the identified molecule may be of great biotherapeutic potential for the development of novel antimicrobial reagent.

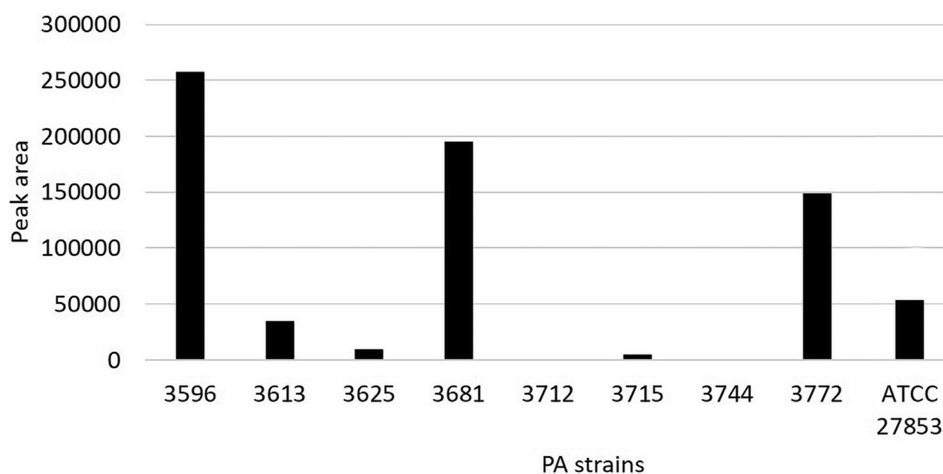

Fig. 5. The relative concentration of PCN in the cell-free culture medium of the nine PA strains.

Many mechanisms have been proposed regarding competition and inhibition among bacteria. In relation to PA strains, pyocins (polypeptide toxins) are believed to be one of the principal mechanisms underlying the antibacterial activity.[23,24] In addition, phenazine and its derivatives secreted by PA, including pyocyanin (PYO), PCN, 1-hydroxyphenazine (1-HP) and phenazine-1-carboxylic acid (PCA), are considered as virulence factors against many bacteria and fungi.[25] Cardozo et al. reported that PCN extracted from the cultural medium of PA reveals growth inhibitory activities against MRSA.[15] Furthermore, a marine PA strain, showing an inhibition capability against the marine pathogen, *Vibrio anguillarum*, can produce PCA as the effective compound.[26] It was demonstrated that PCA causes accumulation of reactive oxygen species (ROS) and lysis of *V. anguillarum*.[26] Furthermore, four phenazine compounds, PYO, PCN, 1-HP and PCA secreted by PA induced retarded growth and swollen conidia of the fungus *Aspergillus fumigatus*, via the production of ROS and reactive nitrogen species (RNS), resulting in cell damages and death of the target organisms.[25] Recently, Xu et al. reported that secondary metabolites extracted from the cell-free supernatant of PA culture showing a broad antimicrobial spectrum against MRSA, VISA and fungi, are distinct from PCA and pyocyanin, further suggesting the diversity of antimicrobial components produced by PA.[16]

The assessment of the inhibitory activity of the PA strains against the AB strains showed that the four PA strains (PA3596, PA3681, PA3772, and ATCC27853) that exerted a high inhibitory activity against AB also had a high amount of PCN as assessed by LC/MS. Further, the dose-dependent effect observed with PCN on the inhibitory zones suggests that PCN exerts inhibitory activities against all the five AB strains used in this study. The results demonstrated the capacity of clinical strains of PA in inhibiting the growth of MDR-AB and AB strains, with PCN possibly being the inhibitory agent responsible for the action.

In this study, we demonstrated that PCN may be the major inhibitory biological agent responsible for the inhibitory activity. However, we do not exclude the possibility that other chemicals produced by PA have inhibition activity against MRSA, as reported by Xu et al.[16] may be capable of inhibiting AB, since two AB strains, AB3712 and AB3715, with undetectable amount of PCN in culture medium still have a low inhibitory activity (Fig. 5 and Table 2).

In this study, none of the cell-free culture medium of the nine PA strains showed inhibition zones against AB3748, probably because the strain is more resistant to PCN than the other four AB strains. It is hypothesized that during the 16-h incubation period many PA produced inhibitory substance(s) and secreted out of the cell. The majority of the inhibitory substance(s) were bactericidal instead of bacteriostatic, in most of the cases an individual PA against individual AB was examined, the diameters of the inhibition zones were about the same with the 16, 22, and 45-h incubations. The supernatants of PA strains tested were from the 16-h incubation. However, when the results of the colony plates were examined, many of the nine PA strains showed inhibition against all five AB strains, and the diameters of the inhibition zones of the increased as the incubation time increased, indicating that the PA strains continued to produce the inhibitory substance(s) during the 45-h incubation period.

PA06 and PA46 strains have been reported to have strong antimicrobial capacities.[16] The small molecules produced by PA06 and PA46 have been reported to be functionally similar to phenazine-1-carboxylic acid and pyocyanin. These also exert potential antimicrobial activities not only against MRSA but also vancomycin intermediate-resistant *S. aureus*, *Corynebacterium* spp., *M. catarrhalis, Candida albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*.[16] Although Xu et al. reported that these molecules were ineffective against *A. baumannii*,[16] this discrepancy with our results may be due to the difference in the bacterial strains used in both studies.

The inhibitory activity exerted by the selected strains of *P. aeruginosa* in this study may also be partly due to other competitive substances such as bacteriocin and antimicrobial peptides as these are bactericidal to multidrug-resistant bacteria.[27] Although *A. baumannii* and *P. aeruginosa* are associated with intrinsic resistance to antibiotics,[1] the observation that specific components (e.g., PCN) of the clinical strains of *P. aeruginosa* exert inhibitory actions in multidrug-resistant *A. baumannii* is of great clinical importance. This idea suggests the possibility of developing

effective therapeutic agents based on these components. One area where the use of PCN and other components of PA may be of critical importance is in the intensive care unit (ICU) settings. In Taiwan, for instance, AB is one of the principal culprits associated with multiple drug resistance, thereby complicating the clinical outcomes of adults and neonates at ICUs.[28,29] In addition, the rapid increase in MRSA and MDR-AB in surgical ICUs[3] implies that PA-derived PCN are potentially viable disinfectants. Interestingly, the prior identification and characterization of gene clusters for the synthetic pathways of PCN and pyocyanin in PA[14,30] will provide a platform for the mass production of PCN via genetically engineered PA for therapeutic and/or disinfecting purposes.

The PFGE results shows that no significant difference(s) between the *Spe*I-PFGE patterns of PA3772 and PA3715 (Fig. 2a). However, PA3772 showed a high antimicrobial activity, whereas the activity of PA3715 was much weaker (Table 2). We therefore performed PFGE for these two strains using another PFGE program and different *Spe*I-PFGE patterns were observed: PA3772 had an extra150 Kbp *Spe*I-fragment whereas PA3715 had an extra 40 Kbp *Spe*I-fragment (data not shown). It is thus speculated that gene(s) responsible for AB inhibition or PCN biosynthesis/secretion is localized in this 150 Kbp *Spe*I fragment in PA3772, which is missing in PA3715. Excluding these two strains, the results of the PFGE analysis shows there is no cluster phenomenon in *P. aeruginosa* showing the inhibitory activity against *A. baumannii*, suggesting that the observed inhibition may not be due to horizontal gene transfer but vertical transfer mechanism. This may be attributed to the ability of the *P. aeruginosa* strains exhibiting significant variability in pathogenicity and ecological flexibility.[31] If this is true, understanding the exact mechanisms how the vertical transfer occurs will be crucial in developing any clinically viable agent against multidrug-resistant *A. baumannii*.

In conclusion, this is the first study, as far as we are aware, to report that selected strains of *P. aeruginosa* can exert inhibitory actions on multidrug-resistant clinical isolates of *A. baumannii*. PCA may be the major inhibitory agent responsible for this biological phenomenon. Although this finding raises the possibility of developing effective therapeutic antibiotics from specific components of *P. aeruginosa* for the treatment of *Acinetobacter*-associated infections and potentially viable disinfectant for use in hospital settings such as ICUs, the possible mechanisms through which the candidate molecules exert their antimicrobial activity need to be further investigated, especially at the molecular level.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Review Board of Taichung Hospital with the approval number IRB-05-06.

Declaration of competing interest

All authors declare that they have no conflicts of interest related to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2020.11.006.