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Comparative Phenotypic and Genotypic Properties of *Pseudomonas aeruginosa* Isolates from Clinical and Environmental Sources

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ABSTRACT

Objective: To provide a better understanding on the potential threat of *P. aeruginosa* as a whole, this study was conducted to compare the phenotypic and genotypic characteristics between isolates from the two different sources.
Method: Twenty isolates were successfully harvested from soil while 30 clinical isolates were viably available in collection for comparison. Several biochemical tests and 16S rRNA DNA sequence homology were conducted for species identification. Pigmentation, antimicrobial susceptibility pattern and virulence genes distribution were determined and correlated in 2x2 contingency tables. BOX- and ERIC-PCR were done to identify the genetic relationship among the 50 isolates.
Result: All soil isolates showed only pyoverdine pigment production while clinical isolates produced either pyocyanin or pyoverdine, or both pigments. Soil isolates were largely susceptible to the tested antibiotics while antimicrobial resistance as well as multidrug resistance was observed among clinical isolates. Virulence genes were differentially detected in both clinical and soil isolates with *exo*S being the most dominant in both groups of isolates. Composite analysis of BOX-ERIC-PCR indicated all isolates to have no close genetic relationship.
Conclusion: Soil isolates are largely susceptible to antibiotics but do carry important virulence genes and pyoverdine. The later was reported to be associated with biofilm capacity which may facilitate survival of isolates in environment as well as during human infection. Therefore, both groups of isolates may pose infection threat at various degrees.
Keywords: *Pseudomonas aeruginosa*; pigmentation; virulence genes; antibiotic susceptibility; composite BOX-ERIC-PCR.

1. Introduction

Pseudomonas aeruginosa is widespread in nature inhabiting various environmental niches such as surface, soil and water (Ryan and Drew, 2010). It can grow and multiply in almost any moist environment with minimal nutritional requirements. *P. aeruginosa* naturally develops several intrinsic abilities such as antibiotic resistance, biofilm formation and various secretory virulence factors (Landman and Quale, 2002; Lau *et al*., 2004). These characteristics provide the advantage for *P. aeruginosa* to be a potential opportunistic pathogen (Van Delden and

Iglewski, 1998; Wunderink and Mendoza, 2007). In hospital settings, *P. aeruginosa* can cause uncontrolled nosocomial infection causing diseases such as pneumonia and blood infections (Landman and Quale, 2002; Lau *et al*., 2004; Martin and Yost, 2011). The major risk groups are those with wounds and immunocompromised condition (Van Delden and Iglewski, 1998; Wunderink and Mendoza, 2007).

The pathogenicity of *P. aeruginosa* is closely associated with intrinsic virulence factors comprising production of various pigments, extracellular toxins and secretory enzymes (Apodaca *et al*., 1995). Pigments have been shown to have direct and indirect roles in disease pathogenesis. Pyocyanin and pyoverdine are two common pigments produced by *P. aeruginosa*. A recent study demonstrated that pyocyanin had a direct fast killing on nematode (*Caenorhabditis elegans*) through reactive oxygen production (Mahajan-Miklos *et al*., 1999). Pyoverdine was also previously reported to have an indirect role in virulence by competing for iron with human iron-binding protein. Consequently, this leads to cell death because human cell fails to transport nutrition from cell-surrounding due to loss of iron that usually binds tightly with human transport protein (Meyer *et al*., 1996). Several toxins are also secreted by *P. aeruginosa* referred as exotoxins (Hauser *et al*., 1998). For example, exoenzyme S which is encoded by *exo*S gene, is recognized as GTPase-activating protein (GAP) and ADP-ribosyltransferase (ADPRT), acting as bifunctional enzymes. It is secreted directly into the cytosol of epithelial cells and disrupts the normal organisation of eukaryotic cytoskeleton (Shaver and Hauser, 2004). Another exotoxin is recognized as exoenzyme U, encoded by the *exo*U gene, and responsible in destroying eukaryotic cell membrane (Tamura *et al*., 2004).

Elastase protein, encoded by *las*B is a metalloproteinase enzyme secreted by *P. aeruginosa* into the extracellular space and is responsible in degrading lung elastin tissue leading to reduced lung elasticity (Alcorn and Wright, 2004). Gene *plcH* encodes for phospholipase C which is one of phospholipase family secreted by *P. aeruginosa* into the extracellular space. Phospholipase C protein targets eukaryotic membrane phospholipids and has been shown to participate in the pathogenesis of *P. aeruginosa* in acute lung injury and inflammation (Wiener-Kronish *et al*., 1993, Konig *et al*., 1997). The formation of mucoid colonies of *P. aeruginosa* is composed of alginates, involving *algD* gene, which will protect the bacterium from host immune response and antibiotics (Govan and Deretic, 1996). While extracellular neuraminidase, encoded by *nan*1 gene, plays an important role in implantation of the bacterium into respiratory epithelial cells (Davies *et al*., 1999).

Although distribution and functions of these genes have been investigated in *P. aeruginosa* in recent years (Idris *et al*., 2012; Fazeli and Momtaz, 2014), the studies mainly focused on clinical isolates. Considering the ubiquity nature of *P. aeruginosa*, the distribution and prevalence of these virulence factors from environmental sources would be of concern as well, as they may also pose infection in the community setting. Comparison of these data between the two sources of isolates would provide a better understanding on the potential threat of *P. aeruginosa* as a whole, particularly those from environment whereby studies are still lacking. Therefore, this study was intended to compare the phenotypic and genotypic traits between isolates from hospital setting and soil as the environmental source.

2. Materials and Methods

2.1. Bacterial isolation

Thirty viable isolates of clinical origin were readily available in stock culture collection, and used in this study for comparison with soil isolates. The clinical isolates were collected in year 2012 and no longer traceable to patients. Each originated from a single different individual and from various clinical specimens; 16 from pus, 5 from blood, 2 from tissue, 2 from sputum and 5 from tracheal aspirate. As for the environmental source, 20 isolates were successfully harvested from respective successive soil samples randomly collected at various recreational and public access areas in Klang valley. The soil samples (10 g) were taken up to a depth of 10 cm. The soils were handled and transferred aseptically using sterile spatula into a sterile plastic container and kept at 4˚C.

Soil samples were subjected to serial dilution (up to10^{-3}) prior to microbial cultivation. One gram of soil sample was mixed well with 9 ml of 0.85% saline solution according to methods described by Wellington and Toth (1994). 0.1 ml of each dilution was spread onto Cetrimide agar (Merck, Germany) and incubated at 37^{o}C for 24 to 72 hrs. Only one single colony from each soil sample was collected, propagated and preserved in Luria Bertani with 20% glycerol at -80°C. Soil isolates were labeled with 'E' (E1-E20) and 'C' (C1-C30) for isolates of clinical origin. *P. aeruginosa* ATCC 27853 was used as a control in all experiments.

2.2. Phenotypic and species identification

All fifty *P. aeruginosa* isolates, including *P. aeruginosa* ATCC27853 were subjected to phenotypic identification by standard bacteriological methods which involved colony morphology, Gram-staining, oxidase and catalase reaction. For further phenotype identification, a single colony of each isolates was streak onto several selective and differential media; MacConkey agar, blood agar, Pseudomonas agar P and Pseudomonas agar F. Positive pigmentation result on Pseudomonas agar P showed blue-green colonies and that on Pseudomonas agar F showed yellowish-green colonies. 16S rRNA gene amplification by polymerase chain reaction (PCR) was further conducted to confirm the species using *P. aeruginosa* specific primers: PA-SS-F; 5'-GGG GGA TCT TCG GAC CTC A-3' and PA-SS-R; 5'-TCC TTA GAG TGC CCA CCC G-3' with the expected amplicon size of ~1 kbp as described by Spilker *et al*. (2004). Genomic DNA of isolates was extracted using GF-1 Bacterial DNA Extraction Kit Version 2.1 (Vivantis Tech., Malaysia). PCR was carried out in a 25µl reaction volume with the following reagents: 12.5 µl of 2x PCR master mix (0.05µ/µl *Taq* DNA polymerase, 2x VibufferA, 0.4 mM dNTP's, 3.0 mM $MgCl_2$) (Vivantis tech., Malaysia), 1 µl each of forward and reverse primers (0.4 µm) and 1µl of template DNA. PCR reactions were carried out using C1000^{TM} Thermal cycler (Bio-Rad Lab. Pty Ltd., Australia). The initial denaturation step was carried out for 2 min at 94˚C, followed by 30 cycles of denaturation step for 2 s at 94^{o}C, the annealing step for 1 min at 50°C, and the extension step at 72.0^{o}C for 30 s. A final extension step was carried out for 7 min at 72.0^{o}C. The PCR products were electrophoresed in 1.5% (w/v) agarose gel at 75V and visualized by Alpha Imager Gel Documentation System (Alpha Innotech, USA) under transillumination of ultra violet (UV) light. Purified PCR products were sent for sequencing for gene homology search.

2.3. Antimicrobial susceptibility assay

Antibiotic susceptibility of the fifty isolates was determined by Kirby Bauer disc diffusion method (CLSI, 2012). Seven different antibiotic disks (Oxoid Ltd., UK) were used: piperacillin-tazobactam (TZP) (100/10 µg), cefepime (FEP) (30 µg), ceftazidime (CAZ) (30 µg), imipenem (IPM) (10 µg), meropenem (MEM) (10 µg), gentamicin (CN) (10 µg) and ciprofloxacin (CIP) (5 µg). Bacterial suspension was adjusted against 0.5 McFarland turbidity standard and spread on Mueller-Hinton Agar (MHA). The antibiotic discs were placed on the plates about 4 to 5 cm apart from each other and incubated at 37°C for 16-18 hrs. Diameter of zone inhibition was measured according to the Clinical and Laboratory Standards Institute Guideline for interpretation of susceptibility categories (CLSI, 2012).

2.4. Amplification of virulence genes by PCR

Amplification of seven virulence genes [exoenzyme S (*exoS*), exoenzyme U (*exoU*), elastase LasB (*lasB*), haemolytic phospholipase C precursor (*plcH*), GDP mannose 6-dehydrogenase (alginate) (*algD*) and neuraminidase (*nan1*)] were conducted by PCR using published primers (Mitov *et al*., 2010). The reaction was carried out in a 25µl total volume per reaction mixture containing 8 µl of 2x PCR master mix (0.05µ/µl Taq DNA polymerase, 2x VibufferA, 0.4 mM dNTP's, 3.0 mM $MgCl_2$) (Vivantis tech., Malaysia), 0.625 µl each of forward and reverse primers (0.25 µm) and 3 µl of genomic DNA. The amplification reaction was set at initial denaturation for 5 min at 94˚C, followed by 30 cycles of denaturation step for 40 s at 94˚C, annealing step for 60 s at 57˚C for *exoS, exoU, lasB* and *plcH*; 54˚C for *algD*; 49˚C for *nan1*, and extension step for 90 s at 72˚C, and a final extension for 7 min at 72˚C. PCR reactions were carried out using C1000^{TM} Thermal cycler (Bio-Rad Lab. Pty Ltd., Australia). The PCR product were electrophoresed in 1%-1.5% (w/v) agarose gel at 75-100V and visualized by Alpha Imager Gel Documentation System (Alpha Innotech, USA) under transillumination of UV light. DNA bands were checked for expected size and representatives were subjected to purification and sequencing for gene homology search.

2.5. BOX-PCR typing

BOX-PCR fingerprinting was carried out by using a single primer (5'-CTA CGG CAA GGC GAC GCT GAC G-3') as described by Koeuth *et al*. (1995). The PCR reactions were carried out in a 25 µl total volume per reaction in C1000^{TM} Thermal cycler (Bio-Rad Lab. Pty Ltd., Australia) containing 12.5 µl of 2x PCR master mix (0.05µ/µl *Taq* DNA polymerase, 2x VibufferA, 0.4 mM dNTP's, 3.0 mM $MgCl_2$) (Vivantis tech., Malaysia), 1 µl of specific primer (0.25 µm) and 2 µl of genomic DNA (Dawson *et al*., 2002). The amplification reaction was set at initial denaturation step for 5 min at 94˚C, followed by 35 cycles of denaturation step for 1 min at 94˚C, annealing step for 2 min at 49˚C, and extension step for 2 min at 72˚C, with a final extension of 7 min at 72˚C. Amplified PCR products were electrophoresed in 1.5 % (w/v) agarose gel at 70V and viewed by Alpha Imager Gel Documentation System (Alpha Innotech, USA) under transillumination of UV light.

2.6. ERIC (Enterobacterial Repetitive Intergenic Consensus)-PCR

ERIC-PCR was carried out by using a set of primers [ERIC-1 (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC-2 (5'AAG TAA GTG ACT GGG GTG AGC G-3')] as described by Versalovic *et al*. (1991). The PCR reactions were carried out in a 25 µl total volume in C1000^{TM} Thermal cycler (Bio-Rad Lab. Pty Ltd., Australia) containing 12.5 µl of 2x PCR master mix (0.05µ/µl *Taq* DNA polymerase, 2x VibufferA, 0.4 mM dNTP's, 3.0 mM $MgCl_2$) (Vivantis tech., Malaysia), 1 µl of specific primer (0.25 µm), 2 µl of genomic DNA. The amplification reaction was set at initial denaturation step for 4 min at 94˚C, followed by 38 cycles of denaturation step for 40 s at 94˚C, annealing step for 60 s at 50˚C, and extension step for 3 min at 65˚C. A final extension was set at 10 min at 65˚C. Amplified PCR products were electrophoresed in 1.5 % (w/v) agarose gel at 70V for 75 min and viewed by Alpha Imager Gel Documentation System (Alpha Innotech, USA) under transillumination of UV light.

2.7. DNA fingerprint analysis

DNA fingerprint patterns generated by BOX- and ERIC-PCR were analyzed by BioNumerics version 6.0 (Applied Maths, Kortrijk, Belgium) software via Unweighted Pair Group Mathematical Averaging (UPGMA) and Dice Coefficient. A composite analysis was generated based on DNA fingerprinting pattern from both typing procedures for a higher dendrogrammatic resolution.

2.8. Statistical analysis

Data from the disc diffusion tests were assigned into 3 categories; resistant, intermediate and susceptible. For simplicity, isolates with intermediate level were grouped as resistant strains. Data on the antibiotic susceptibility, pigmentation profiles and virulence genes distribution were analyzed in 2x2 contingency tables using Chi-square ($\chi 2$) and Fisher's exact tests with $p<0.050$ as the significant level.

3. Results

3.1. Bacterial identification and pigmentation profile

Phenotypic tests showed the expected outcome for *P. aeruginosa*. All isolates were able to grow on Cetrimide agar, a selective medium for pseudomonas, lactose fermenters based on the yellow appearance on MacConkey agar, gram negative rod, oxidase and catalase positive. Expected amplicon size of 16S rRNA gene sequences (~1.0kbp) were successfully obtained with 100% sequence homology in BLAST search confirming the *P. aeruginosa* species (result not shown). Pigmentation profile was determined based on growth on Pseudomonas agar P and F as shown in Table 1. All *P. aeruginosa* isolates from soil samples showed no pigmentation on Pseudomonas agar P but almost all showed a positive result on Pseudomonas agar F except isolate E3 (no pigmentation on both agar).

On the other hand, a variable pigmentation profile was observed for clinical isolates whereby a majority of them (n =20) showed positive pigmentations on both Pseudomonas agar P and F, while five isolates showed a positive result on Pseudomonas agar F but a negative result on Pseudomonas agar P, and four isolates showed a positive result on Pseudomonas agar P but a negative result on Pseudomonas agar F. One isolate showed negative result on both Pseudomonas agar (Table 1). Chi-square (χ^2) and Fisher's exact tests indicate that only tabulation on Pseudomonas agar P had significant correlation ($p < 0.001$), which was expected as none of soil isolates showed pigmentation on agar P, thus displaying an obvious difference between the two groups of isolates.

Table 1. Pigmentation pattern of the fifty *P. aeruginosa* isolate on Pseudomonas agar P and F.

Pseudomonas agar	Isolate	Positive Pigmentation
P	Soil (n=20)	0
	Clinical (n=30)	24(80%)
F	Soil (n=20)	19(95%)
	Clinical (n=30)	26(86.7%)

3.2. Antimicrobial susceptibility pattern

In the antimicrobial assay, an obvious difference was observed that all soil isolates were largely susceptible to all tested antibiotics except one isolate against meropenem. For clinical isolates, a variable resistance pattern was observed towards the different antibiotics; piperacillin-tazobactam (8 isolates), gentamicin (8 isolates), ciprofloxacin (9 isolates), ceftazidime (7 isolates), imipenem (6 isolates), meropenem (3 isolates), cefepime (6 isolates), (Figure 1).

Multi-drug resistant isolates (MDR), defined as resistance to at least two pseudo-antimicrobial classes

(Mohanasoundaram, 2011), covered 27% (8/30) among the clinical isolates, labeled with C16, C17, C18, C19, C25, C26, C27 and C29. Chi-square (χ^2) and Fisher's exact tests indicate a significant correlation ($p<0.05$) between clinical and soil isolates which was expected due to the large antibiotic susceptible isolates from soil.

3.3. Distribution of virulence genes

Six virulence-associated genes: *exoU*, *exoS, lasB, plcH, algD* and *nanI* were screened in this study among the isolates due to their established roles in pathogenesis. Expected amplicon size for the respective genes was successfully obtained in some isolates indicating their differential pattern of distribution in different isolates. The identity of the amplicon was confirmed as the respective targeted genes by sequencing (data not shown).

For clinical isolates, the presence of *lasB* gene was the highest (100%), followed by *exoS* (93%) *plcH* (93%), *algD* (77%), *exoU* (37%) and *nanI* (17%). For soil isolates, *exoS* gene was the highest in presence (85%), followed by *lasB* (70%), *algD* (60%), *plcH* (45%), *exoU* (45%) and *nanI* (25%). *ExoS* gene had the highest percentage of presence in both groups of isolates (90%), followed by *lasB* (88%), *plcH* (74%), *algD* (70%), *exoU* (40%) and *nanI* (20%) (Figure 2). Chi-square (χ^2) and Fisher's exact showed a significant correlation ($p<0.050$) only for genes *exoS*, *lasB* and *plcH* between the clinical and soil isolates (Table 2).

3.4. DNA fingerprint outcome

BOX- and ERIC-PCR generated DNA fingerprint from three to thirteen bands with size ranging from 250 bp until 10,000 bp among the fifty *P. aeruginosa* isolates. The composite analysis of the DNA banding pattern was correlated with the isolation source, distribution pattern of antimicrobial susceptibility and virulence genes for potential correlation. Nevertheless, the outcome of the generated dendrogram showed a genetically diverse tabulation of the isolates without any obvious correlation in relation to the studied characteristics of the isolates (data not shown).

As shown in Figure 3, none of the isolates were clustered at genetic similarities of more than 80%. One big cluster appeared only at a 40% genetic similarity labelled with 'A' with no specific pattern in the tabulation of isolates in relation to the source of isolation, antimicrobial susceptibility and virulence gene distribution. This may reflect a collection of genetically distinct isolates and thus the distribution of the virulence genes and antimicrobial resistance are presented in different isolates rather than a group of related strains which may potentially be carrying some common antimicrobial resistance and/or virulence traits.

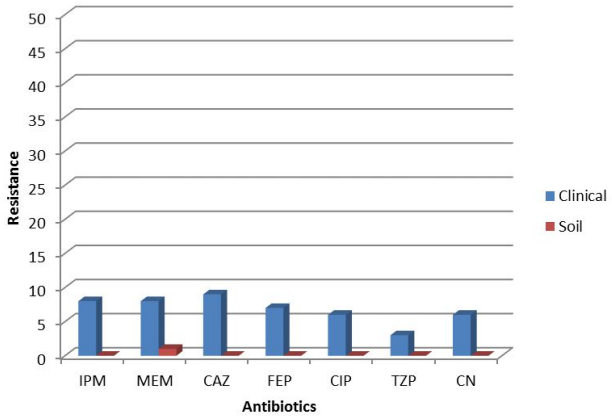

Figure 1. Antimicrobial resistance pattern of the fifty *P. aeruginosa* isolates. Blue: clinical isolates. Red: soil isolates.

Table 2. Virulence genes distribution in relation to soil and clinical isolates with significant tabulation ($p<0.050$)

Genes	***lasB*+ (n=44)**	***plcH*+ (n=37)**	***exoS*+ (n=45)**
Soil (n=20)	14(70%)	9(45%)	17(85%)
Clinical (n=30)	30(100%)	28(93%)	28(93%)

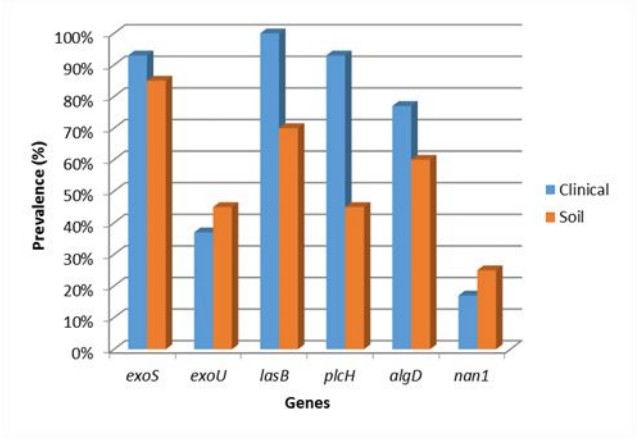

Figure 2. Distribution of virulence genes among fifty *P. aeroginosa* isolates. Blue: clinical isolates. Red: soil isolates.

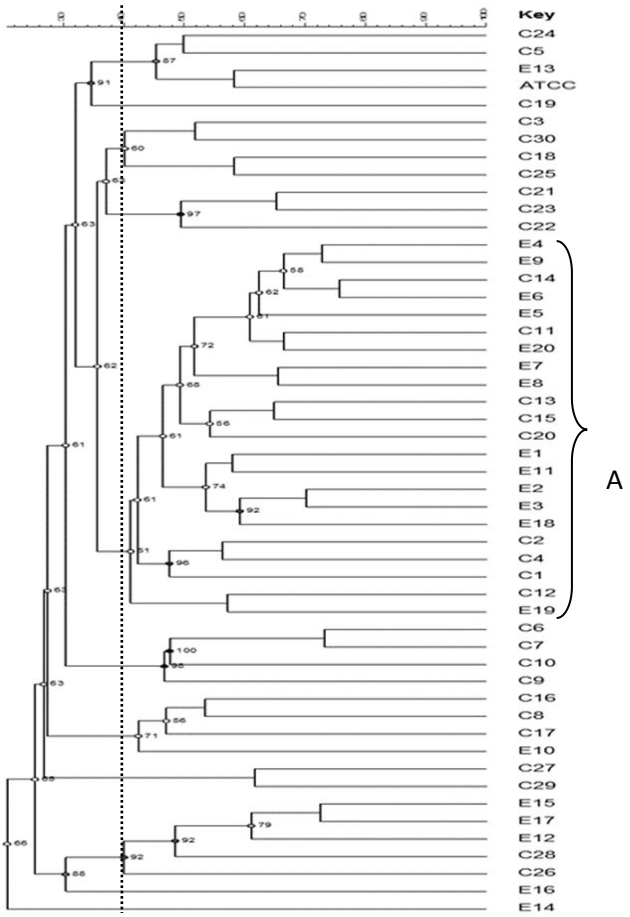

Figure 3: A dendrogram of composite BOX-ERIC-based genetic relation among the fifty *P. aeruginosa* isolates indicating a big cluster (A) at a 40% similarity. C: clinical isolates, E: soil isolates.

4. Discussion

Pseudomonas agar P and F are recognized as fluorescein enhancer and also differential agar media for *P. aeruginosa* pigmentation. For the later, *P. aeruginosa* can secrete a variety of pigments, and Pseudomonas agar P and F were developed to test the ability of *P. aeruginosa* to produce pyocyanin (blue-green colonies) and pyoverdine (yellowish-green colonies).

In this study, a majority of both clinical and soil isolates produced pyoverdine and all soil isolate showed only pyoverdine pigmentation. For the clinical isolates, a large proportion (67%) showed both pyocyanin and pyoverdine pigmentations, while the rest produced either pyocyanin or pyoverdine, and only one isolate did not produced both pigments. Pigmentation pattern may serve as the indicator to distinguish virulence potential of *P. aeruginosa* as production of pyocyanin pigment is associated with the major virulent factors such as elastase, protease, siderophores, DNase and twitching motility of *P. aeruginosa*. (Finlayson and Brown, 2011). Unfortunately, all soil isolates did not exhibit the pigmentation except for pyoverdine. Nevertheless, a previous study reported that pyoverdine acted as a strong metal chelator by removing iron from human ferritransferrin rapidly (Meyer *et al*., 1996). In addition, another study suggested that biofilm formation in *P. aeruginosa* required pyoverdine-mediated iron transport.

Mutant *P. aeruginosa* with deficient pyoverdine production was disturbed in biofilm development and addition of pyoverdine restored back the biofilm formation capability (Patriquin *et al*., 2008). Interestingly, *P. aeruginosa* from harsh environment condition such as rock and soil was reported to have a high survival rate because of biofilm formation by producing pyoverdine (Glick *et al*., 2010). Biofilm formation was also associated with the survivability of *P. aeruginosa* during infection in human as well (Yan *et al*., 2008). Therefore, it could be inferred that *P. aeruginosa* from soil can emerge as a potential virulent strain as well because of the production of pyoverdine pigment.

The incidence of antimicrobial resistance among clinical isolates are readily understood due to high selective antimicrobial pressure in clinical setting (Gordon *et al*., 2013). This condition will select resistant isolates to thrive and disseminate. Those in soil might live in a diverse microbial population whereby microbial competition may also exist especially with those of antibiotic-producing organisms such as Streptomyces species, which also live in soil thus leading to selection affecting the soil Pseudomonas species. There are lacks of studies to elucidate the complex microbial interaction in soil but possibly not to the extent as taking place in the clinical setting so that the tendency of isolating high antimicrobial resistant isolates in soil is low. But this study detects one soil isolate (E16) with meropenem resistance. To our knowledge, no previous report is available on soil isolates of *P. aeruginosa* with meropenem resistance. Whether this isolate is due to cross dissemination from other sources such as human is not known.

Elastase (*lasB*) and exoenzyme S (*exoS*)-encoding genes were the most presented virulence genes in both groups of isolates in this study. Elastase is known as a virulence determinant in *P. aeruginosa*. It can degrade epithelial lung tissues by cleaving pulmonary surfactant protein D, and thus making lung to lose its elasticity (Alcorn and Wright, 2004). The role of exoenzyme S was associated with intermediate virulent effect during lung infection and when

tested in mouse model of acute pneumonia (Shaver and Hauser, 2004). However, our result showed that the presence of *exoS* gene was higher in soil isolates among other genes as compared with presence in clinical isolates. This result is consistent at certain extent with a previous report on the high expression of ADP-ribosyltransferase (protein encoded by *exoS* gene) in environmental isolates compared with clinical ones. Therefore it is suggested that, exoenzyme S may play a bigger role in the *P. aeruginosa* fitness in environment and might also be a major factor of transition from soil organism into human pathogen (Ferguson *et al*., 2001; Lomholt *et al*., 2001). Furthermore, another finding reported on the absence of *exoS* structural gene in certain clinical isolates indicating the lesser importance of *exoS* gene in clinical setting (Ferguson *et al*., 2001). Another finding in this study showed a very low presence of *nan1* gene in both groups of isolates. The presence of *nan1* gene was usually detected in isolates from cystic fibrosis (CF) in clinical setting (Dacheux *et al*., 2000). Mitov *et al*. (2010) did detect *nan1* gene in non-CF isolates but only at a low level as compared with that in CF isolates. Although clinical isolates were used in this study, none of them was isolated from case associated with CF to support such an observation.

5. Conclusion

Comparative analysis in medically important organisms may identify potential differences which are useful in providing output for understanding and managing risks. Phenotypic and genotypic comparisons among isolates in this study indicate a few obvious characteristics between those of clinical and environmental origins, particularly in the antimicrobial resistance and pigmentation profiles. These indicate that the two groups of isolates may have a different capacity and risk of infection in human. Nevertheless, with the regimen of virulence genes carried by *P. aeruginosa* regardless of the origin, each strain may have a potential to pose infection threat. This makes *P. aeruginosa* as an opportunistic organism which shoud not be neglected either in hopsital or community setting. Whether there is a relation between pigment production, antimicrobial and virulence genes distribution among *P. aeroginosa* require a further investigation involving a larger sample size.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

ETHICAL ISSUES

None

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