Malaysian Journal of Microbiology, Vol 17(5) 2021, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.201040

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Proteomic profiling of *Acinetobacter baumannii* ATCC 19606 and Malaysian isolated clinical strain AB-13 using 2-dimensional gel electrophoresis

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Received 17 November 2020; Received in revised form 24 June 2021; Accepted 5 August 2021

ABSTRACT

Aims: *Acinetobacter baumannii* has been identified as one of the six most pathogenic bacteria that is the cause of most hospital bacterial infections according to Infectious Disease Society of America (IDSA). These nosocomial pathogens are notorious worldwide due to its ability in causing lethal infections among immunocompromised patients and its resistance to many strong antibiotics. This study aims to compare the expressed proteins of two *A. baumannii* strain, ATCC 19606 and a pathogenic clinically isolated strain known as AB-13.
Methodology and results: AB-13 clinically strain was isolated from the lower respiratory tract of a patient with pneumonia. In this study, the proteomic profile of both ATCC 19606 and AB-13 are produced using 2-dimensional gel electrophoresis. The total protein contents were extracted, quantified and separated using 2-DE with a pH range of 4-7 to acquire the proteomic profile for comparison. The final analytical gel was analysed using Delta2D software and among the 324 protein spots successfully resolved, 10 spots exhibited signs of differential expression with 7 spots found to be downregulated and 3 spots upregulated (p< 0.01). These differences could signify the evolution AB-13 has undergone as it acquires traits ultimately aiding in its survivability, antimicrobial resistance and pathogenicity within varied environments especially during infections.
Conclusion, significance and impact of study: These findings support the presence of variation in AB-13 from a proteomic perspective, highlighting the pathogen's evolution improving survivability and pathogenicity, warranting in-depth exploration towards understanding *A. baumannii* virulence and pathogenicity.

Keywords: Acinetobacter baumannii, nosocomial infection, 2-DE, proteomic, pathogenicity

INTRODUCTION

Among the most common Gram-negative bacteria that is associated with causing hospital-acquired infections in the USA and the world, *Acinetobacter* spp. ranks top 6 (Peleg and Hooper, 2010; Antunes *et al.*, 2014). One species that stands out within the *Acinetobacter* genus is *A. baumannii* acquiring the title of 'successful global pathogen' (Peleg *et al.*, 2008). If left untreated, these infections can result in a mortality rate as high as 95%, signifying the deadliness of these bacteria (Choi *et al.*, 2016). Global attention towards this bacterium started with a series of infection outbreaks occurring in army triages and hospitals among wounded US soldiers in the Iraq and Afghanistan conflict back in 2001-2003 resulting in a global epidemic in the past 2 decades as more and more hospitals started reporting multiple occurrences of *A. baumannii* outbreaks especially within the ICU wards (Dijkshoorn *et al.*, 2007; Petersen *et al.*, 2007; Peleg and Hooper, 2010).

Widely labelled as a nosocomial pathogen, *A. baumannii* is commonly found in hospital or clinical settings whereby it acts as an opportunistic bacterium and infects patients with vulnerable health or compromised immune system (Nasr, 2020). These bacteria can withstand harsh environments and tolerate antibiotic treatments, owing to its many elaborate defence mechanism. Clinical manifestations include pneumonia,

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

septicaemia, meningitis, urinary tract infections and many more depending on the site of infection. Patients with long hospitalisation stay or are inserted with intubation are at greater risk of acquiring an infection compared to healthy patients (Eliopoulos *et al.*, 2008).

Despite being known for more than 100 years, *A. baumannii* has never posed a serious threat until the introduction of antibiotics in the late 1920s (Alsan and Klompas, 2010). It is believed that the extensive use of antibiotics has ultimately led to this escalating crisis as these bacteria becomes more resistant to many strong antibiotics due to resistance gene transfer and as part of a global response to DNA damage (Norton *et al.*, 2013). Subsequently, mortality rate is increasing as *A. baumannii* infections are extremely difficult to treat if not impossible. Indeed, these bacteria are an old friend, but a new enemy as pointed out by Towner (2009). With strains being either multi-, extensive- or pan- drug resistance *A. baumannii* on the rise, health care personnel are forced to turn to last resort antibiotics such as colistin to treat patients (Paterson and Lipman, 2007). The use of these strong antibiotics may be a double edge sword because apart from killing off the bacteria, it can also cause negative repercussions in patients such as toxicity poisoning in kidneys, liver and even neurons. Besides gaining resistance to antibiotics, *A. baumannii* has other virulence traits such as specific efflux pumps, biofilms, outer membrane proteins and production of enzyme which not only supports the breaking down of antimicrobial agents but can interact with host cells leading to apoptosis (Eliopoulos *et al.*, 2008; Harding *et al.*, 2018). Ultimately, all these mechanism aids the bacteria in adapting to any environmental conditions and overcoming its pressures, thereby, increasing its survivability and posing as a major problem for health care professionals. In the event this trend continues with no other options or cure available to tackle these bacteria, it would put the community and people in an extremely perilous position should there be a sudden epidemic outbreak in the future, where all antibiotics are already exhausted and ineffective against *A. baumannii* infections.

Currently, proteomic studies on *A. baumannii* bacteria have not been fully explored due to the lack of emphasis given on these pathogenic bacteria. The current knowledge of *A. baumannii*'s pathogenesis has been steadily growing but is still limited. Furthermore, these gaps in the proteome research for *A. baumannii* poses significant challenges to the understanding of the pathogen. Through preceding studies, it was shown that *A. baumannii* AB-13 strain is more virulent compared to a less virulent reference strain, ATCC 19606 (Nathan *et al.*, 2016). In a study conducted by Nathan *et al.* (2016) locally isolated *A. baumannii* clinical strains were characterized among them four were multi-drug resistant. Interestingly, the four strains though isolated from different infection sites exhibited similar characteristics and were found to also be of the same grouping when characterize under multi-locus sequence typing (MLST) to be of ST-6. This group within MLST classification were first identified back in 1997 in Spain (Bartual *et al.*, 2005). Furthermore, virulence characterization was also conducted on all the 17 strains using Polymerase Chain Reaction (PCR) to identify the presence of four genes namely, *csu*E, *pga*A, *omp*A and *bau*A. The four strains found to be MDR were also found to possess the 4 virulent genes and among them is the strain AB-13. A summary of the study's results can be found in Table 1. However, this study does not analyse their proteome differences. Only very few studies focus on their molecular mechanism that is their differentially expressed proteins to correlate to the bacterial virulence and pathogenesis.

Therefore, this variation poses an interest as to what causes this significant difference of bacteria virulence between the two bacterial strains although both are of the same species. Hence, with that in mind, this explorative research in proteomic studies on their protein profile is anticipated to shed some light and pave the way for future studies in understanding these bacteria fully, albeit using different clinically relevant strains of *A. baumannii* with suspected difference in virulence.

Table 1: Locally isolated clinical *Acinetobacter baumannii* strains with their year of isolation, source site, MLST ST group, resistance characterization and virulence characterization carried out by Nathan *et al.* (2016) in a previous study.

Strain	Year of isolation	Source	ST	Resistotype[a]											Virulotype[b]			
				AMS	CAZ	IMI	MRP	PB	CN	AK	TGC	CIP	LEV	SXT	*csu*E	*pga*A	*omp*A	*bau*A
AB-7	2012	Wound swab	6	R	R	R	R	S	R	R	S	R	R	R	+	+	+	+
AB-8	2012	Wound swab	6	R	R	R	R	S	R	R	S	R	R	R	+	+	+	+
AB-13	2012	Tracheotomy site	6	R	R	R	R	S	R	R	S	R	R	R	+	+	+	+
AB-14	2013	Wound Swab	6	R	R	R	R	S	R	R	S	R	R	R	+	+	+	+

[a] Resistotype against a panel of 11 known antibiotics were determined using the broth microdilution method. AMS, ampicillin/sulbactam; CAZ, ceftazidime; IMI, imipenem; MRP, meropenem; PB, polymyxin B; CN, gentamicin; AK, amikacin; TGC, tigecycline; CIP, ciprofloxacin; LEV, levofloxacin; SXT, trimethoprim/sulfamethoxacole. S, susceptible; R, resistant.
[b] PCR results using primers *csu*E, *pga*A, *omp*A and *bau*A genes. -, absent; +, presence.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.201040

MATERIALS AND METHODS

Bacterial strain and growth condition

Acinetobacter baumannii ATCC 19606 (ATCC, USA) was taken from existing in-house stock thawed gradually by placing the –80 °C stock into –20 °C freezer the day before, followed by a 4 °C chiller prior to usage. *Acinetobacter baumannii* strain designated AB-13 is a locally isolated strain that exhibited pathogenic and anti-microbial properties (Nathan *et al.*, 2016). The genus *Acinetobacter* was identified using phenotypic markers along with Microflex matrix assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) bench-top (Bruker Daltonik GmbH, Germany) at High Impact Research, University of Malaya, Malaysia. The AB-13 isolate was isolated from the sputum of a hospitalized patient in a tertiary-care hospital in Subang Jaya, Selangor state of Malaysia (Nathan *et al.*, 2016). Both ATCC and AB-13 strain were grown on nutrient agar at 37 °C for 16 h prior to subculturing in 10 mL Luria-Bertani (LB) broth (BD, USA) incubated at 37 °C under 200 rpm for 16 h prior to use. Bacterial cells were centrifuged under 4000 rpm at 37 °C for 5 min and resuspended in PBS twice before being diluted with PBS to form a suspension equivalent to 0.5 McFarland Standard (~1×10^8 CFU/mL) with an absorbance of 0.08–0.1 at 600 nm using a spectrophotometer (ThermoFisher Scientific, USA). Once the ideal suspension is achieved, 1 mL of the suspension was aspirated into a 15 mL conical flask with 1 mL of RPMI 1640 Medium (Corning, USA) and incubated at 37 °C under 200 rpm for 12 h.

Protein extraction and quantification

The bacterial cells were centrifuged under 4000 rpm at 37 °C for 20 min before being resuspended with ultrapure deionised (18.2 MΩ) water. The washing step was repeated thrice, before resuspension and transfer into a fresh 1.5 mL microcentrifuge tube. For protein extraction, ReadyPrep™ Protein Extraction Kit (Total Protein) (Bio-Rad, USA) was used. The procedure was carried out as per the manufacturer's instruction. Briefly, rehydration/sample buffer were prepared fresh and 986 µL was added with 10 µL of tributylphosphine (TBP) and 5 µL of 40% Ampholyte, making up a final volume of 1000 µL known as Complete buffer. The Complete buffer was added to the cells and mixed thoroughly before being centrifuged at ~16,000× *g* for 30 min at a pre-set temperature of 20 °C in order to pellet the cell debris. The supernatant was extracted and transferred into a clean microcentrifuge tube and stored in –80 °C freezer for future use. Quantification of protein samples were carried out using RC DC Assay (Bio-Rad, USA) as stipulated on the manufacturer's manual.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2-DE) was carried out as outlined by manufacturer's manual by Bio-Rad, USA which was adapted from Görg *et al.* (2004) with several minor modifications. Previously extracted protein samples were taken out from –80 °C storage and allowed to thaw before being dissolved in rehydration/sample buffer (8 M Urea, 2 mM TBP, 4% CHAPS, 40% Ampholytes, 0.0002% Bromophenol blue). The sample of 125 µL protein was then loaded onto the IPG strips for passive rehydration of 16 h. The run was of an analytical nature, which used a 7 cm, ReadyStrip pH 4-7, NL IPG strip. Isoelectric focusing were carried out using PROTEAN IEF cell (Bio-Rad, USA) under the following conditions: 0 V start voltage, Ramp to Rapid with Volt-hours of 8–10,000 V-hr, 4,000 V end voltage, temperature 20 °C and maximum current of 50 µA/IPG strip for approximately 6 h. The strips were directly processed or stored in –80 °C for future analysis.

Focused strips were then treated with equilibrium buffer (6 M urea, 2% SDS, 0.05 M Tris/HCl buffer, 20% Glycerol) containing 2 % DTT for the reduction of the proteins followed by 2.5% iodoacetamide within the same equilibrium buffer for alkylation purposes. Both buffers were created fresh and were carried out at room temperature 20 °C for 10 min under gentle agitation. The equilibrated strips were then placed on top of 12.5% SDS-PAGE gel and sealed with Overlay Agarose (Bio-Rad, USA). Second dimension electrophoresis was performed using the Mini-PROTEAN™ Tetra Vertical Electrophoresis Cell (Bio-Rad, USA) at 200 V constant until the dye reached the bottom of the gels. This is followed by Silver staining using the Silver Stain Plus™ (Bio-Rad, USA) comprising of 4 steps, fixative, rinse, staining and development followed by stop step. All steps were carried out as stipulated by the manufacturer's manual. Triplicate runs were carried out for both experimental groups to ensure reproducibility of the experiment.

Gel documentation and analysis

Gel image was acquired using the Densitometer GS-800 ™ (Bio-Rad, USA) with settings set accordingly for all gel imaged. All experimental group triplicate gel images were analysed using the 2D gel analysis software Delta2D (DECODON GmbH, Germany) version 4.7. Briefly, the images of all 6 gels for two experimental groups were loaded into the software and grouped together. The software then analyses the gels in their respective experimental groups to form a proteome map with the fusion of all triplicate gels forming a master gel. Comparison between the AB-13 and ATCC 19606 proteome map was analysed via the software using the built in function of hierarchical clustering, t-test and heat maps to test the relationship between both experimental groups in terms of protein expression profiles for each significant spot.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.201040

RESULTS

2-DE proteome map

The gel images were collectively analysed by the Delta2D software to form a master gel. The master gel acts as a reference gel also known as the proteome map with all the protein spots identified can be seen in Figure 1. A total of 342 protein spots were detected and verified by the software. The representative gel stained with silver for control ATCC 19606 and AB-13 can be found in Figure 2.

Hierarchical clustering and student's t-test analysis

Hierarchical clustering is one of the first profile analysis that was done to test the quality and similarities of the gels produced within their respective group of ATCC 19606 and AB-13. Hierarchical clustering works in the Delta2D by analysing all the six gels together and determining if the sample gels are closely related or have

Figure 1: The 2-dimensional gel electrophoresis proteome map and master gel for the experimental setup representing both ATCC 19606 and AB-13 spots. The orange circles denote each individual protein spots detected and verified following the completion of protein spot verification using the Delta2D software.

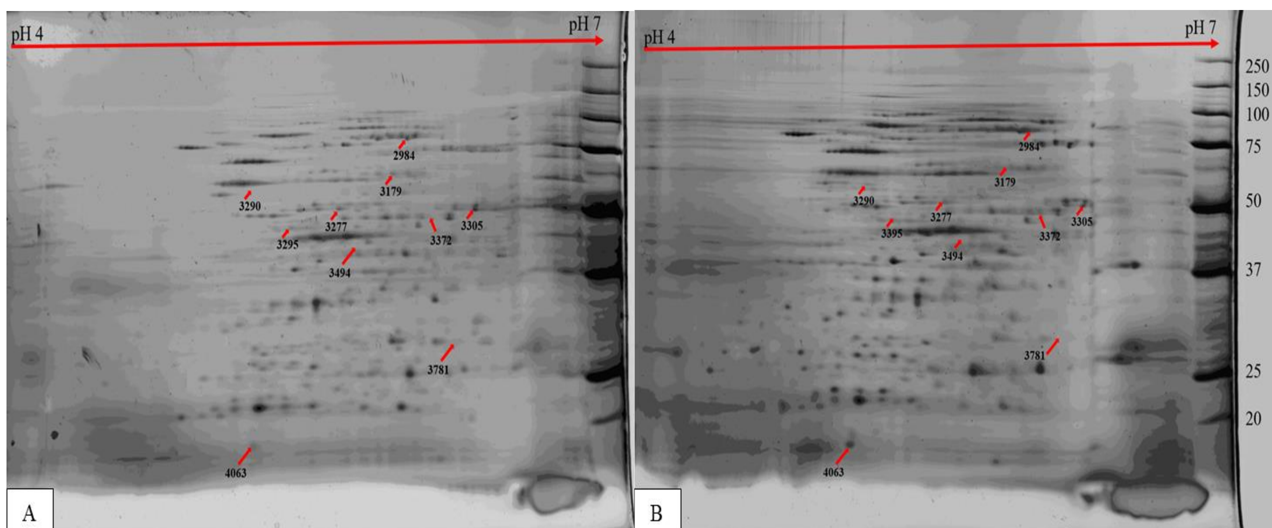

Figure 2: Representative 2-DE maps of proteins extracted from whole cells of *A. baumannii*. A: Control ATCC 19606 strain; B: Multi-drug resistant clinical strain AB-13. Proteins were separated by 2-D gel electrophoresis using pH 4-7 nonlinear IPG strips and 12.5% SDS-PAGE. The number and arrows indicate protein spots identified to be differentially expressed with unique IDs provided in Table 2.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.201040

similar expression levels by clustering the related gels together. The outcome of the hierarchical clustering was as expected theoretically, as the three gels from ATCC 19606 group was clustered together and then colour coded with red. Likewise, the three triplicate gels from AB-13 group was also clustered together and colour coded in green as seen in Figure 3 forming what is known as a heat map. These results also prove that the gels were reproduced consistently from one gel to another and hence, have similar expression levels within their groups.

Additionally, independent sample t-test was used to internally within the software to determine the significant difference between the protein spots among the 2-experimental group. Out of the 342 spots successfully resolved and analysed, 10 were found to be of significant interest (p<0.01). A summary of the significant spots identified can be seen in Table 2 with their respective heat map in Figure 4 detailing their relationship with one another. The protein gel with all 10 differentially expressed spots for control ATCC 19606 and AB-13 can be found in Figure 2.

DISCUSSION

Although we have come a long way since the identification of *A. baumannii* as a significant threat and burden for the healthcare system of the world in recent decades, much of our understanding and knowledge to *A. baumannii*'s virulence traits and pathogenic potential still remains largely elusive (Peleg *et al.*, 2008; Antunes *et al.*, 2014) globally, let alone in Malaysia. Of the 324 protein spots successfully resolved through 2DE gel electrophoresis, ten among them stand out as significant (p<0.01). AB-13 was isolated from a Malaysian university hospital back in 2012 and further our study confirms that from a proteomic angle the expression difference could explain its high resistant to antimicrobial agents and its highly virulent nature. In addition, a recent study (Nathan *et al.*, 2016) revealed that by just sampling one local

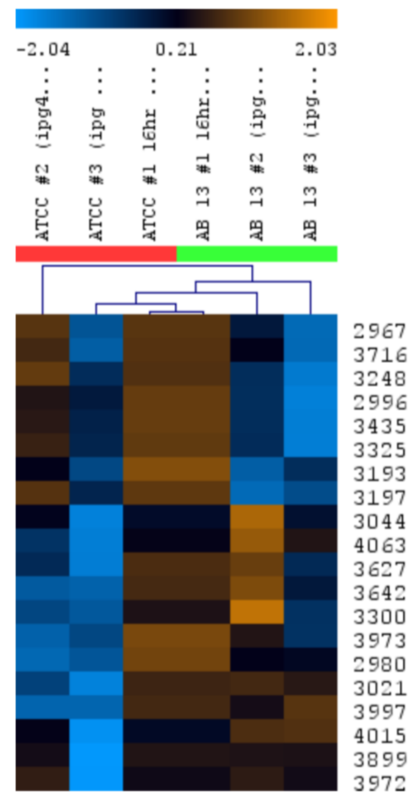

Figure 3: Hierarchical clustering of triplicate gels for ATCC 19606 and AB-13, with the figure representing a selection of a handful of protein spots with their unique ID out of the 342. Spots detected on the master gel and compared with each other. The red subset is for the spots detected on the ATCC 19606 gels and the green subset represent the spots detected on the AB-13 gels. The colours range from brown to blue represent the expression differences of each spot to one another.

Table 2: Estimation of the differentially expressed proteins in ATCC 19606 and AB-13 detected.

Spot ID[a]	Molecular weight (kD)[b]	Isoelectric point (pI)[b]	Expression change[c]
2984	87	6.0	Downregulated
3179	62	6.0	Downregulated
3277	60	6.0	Downregulated
3290	62	5.0	Upregulated
3305	50	6.5	Downregulated
3372	50	6.0	Upregulated
3395	49	5.5	Downregulated
3494	43	5.5	Downregulated
3781	31	6.0	Downregulated
4063	17	5.0	Upregulated

[a] Spot ID numbers corresponds to protein spots comprised in the proteome map.
[b] Molecular weight and isoelectric points are estimated.
[c] Upregulated or downregulated protein spots in AB-13 comparing to ATCC 19606, determined by spot analysis and heat map.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.201040

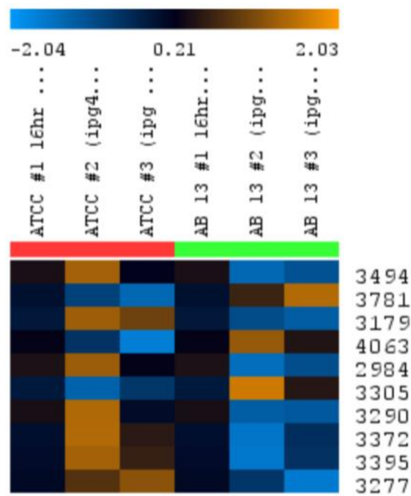

Figure 4: The heat map shows the 10 significant protein spots together with their unique four-digit identification numbers when compared between the two groups, ATCC 19606 and AB-13, as tested at critical *p*-value of 0.01. Each unique ID represents a protein spot across the six gels. Orange colour in the heat map denotes high expression, black colour denotes average expression and blue colour denotes a low expression of protein.

Malaysian tertiary-care hospital in Subang Jaya, in a short 2-year period 23.5% of the *A. baumannii* strains isolated fall under the category of being MDR, resistant to ampicillin/sulbactam, ceftazidime, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin and trimethoprim/sulfamethoxazole. In the same study, usage of multi-locus sequence typing where seven housekeeping genes namely citrate (*gltA*), DNA gyrase subunit B (*gyrB*), glucose dehydrogenase B (*gdhB*), homologous recombination factor (*recA*), 60-kDa chaperonin (*cpn60*), glucose-6-phosphate isomerase (*gpi*) and RNA polymerase sigma factor (*rpo*D), were used to classify the strains according to the detection results of the sequences of nucleic acid from the clinical isolates which were then compared the scheme proposed on the MLST website (http://www.mlst.net) (Ragimbeau *et al*., 2008). These results showed that all the locally isolated MDR strains were of the ST-6 group, which has its clonal lineage to the European Clone II strain responsible for several epidemics across the world and also accounting for 98.9% of *A. baumannii* ICU isolates together with European Clone I in the past 2 decades (Bartual *et al*., 2005; D'Arezzo *et al*., 2009).

When comparing the ATCC 19606 and AB-13 strains among the 10 proteins found to be significantly expressed, 7 were found to be downregulated or under-expressed and the remaining 3 were found to be upregulated or over-expressed. This initially goes against our assumption that a highly virulent and pathogenic strain should have more over-expressed proteins than the control. Several possibilities could be drawn from these observations, firstly, the growth of the bacteria in a rather non-stressful environment undertaken in this experiment resulted in the reduced expression of proteins not required in the given environment. Secondly, acquisition of antibiotic resistance affects the fitness and possibly the virulence of *A. baumannii*. Lastly, genome plasticity of the bacteria could account for the rapid onset of diverse strains in a short period of time which also affects the virulence and pathogenicity of the bacteria.

Proteomic expressions proved the presence of active molecules that will carry out cellular functions for the cell to exhibit its phenotypic properties. This also means that the expression profiles will vary depending on the environmental conditions that exhibit a stimulus to the given pathogen, providing important information about its molecular process that translate into metabolic versatility, varying composition of outer membrane vesicle contents and effects of antimicrobial environments on the bacteria (Fernández-Reyes *et al*., 2009; Soares *et al*., 2009; Hood *et al*., 2010). Our results have shown that even in a similar nutrient rich environment under the same growth conditions, *A. baumannii* ATCC 19606 and AB-13 strains already exhibit variable protein expression from one another. Several studies have shown that under specific stressed conditions, *A. baumannii* will initiate the production of specific systems to assist in its survival under the given circumstances. One such study (Nwungo *et al*., 2011) showed that ATCC 19606 cells cultured under different iron-rich and -chelated conditions resulted in 58 protein spots that were differentially expressed. As iron plays a key role in a diverse number of cellular processes from electron transport, nucleic acid synthesis and free radical protection, it is to no surprise that the ATCC and clinical strains would possess the genes necessary to survive in an iron limiting condition (Weinberg, 2004; Eijkelkamp *et al*., 2011; Gaddy *et al*., 2012).

In addition, proteomic expression has also been known to vary significantly depending on the growth-phase the bacteria are in. When comparing between the three phases exponential, early stationary and late stationary, a study (Soares *et al*., 2010) found that 76 proteins were significantly differentially expressed during *in vitro* growth, broken down to 13 membrane and 63 cytosolic proteins. They observed that a high number of virulence factors were induced as the bacteria progresses from exponential to early stationary phase. Among them were proteins involved in cell adhesion, biofilm formation, such as lipoprotein NlpE and putative peptidoglycan-binding LysM, which have been known to be present and expressed in catheter-related urinary and bloodstream infections (Rodríguez-Baño *et al*., 2008). The lack of which affected *A. baumannii* virulence (Kuo *et al*., 2017). This pattern was also corroborated by transcriptomic analysis showing distinct changes between cells that were planktonic and sessile (Rumbo-Feal *et al*., 2013). As the bacteria progressed through the following growth phases, translation, ribosomal structure and biogenesis saw a stark declined as proteins found to be directly associated with reactive oxygen species ROS metabolism and detoxification process increased. This suggest that as

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(5) 2021, pp. 505-513
DOI: http://dx.doi.org/10.21161/mjm.201040

the bacteria approached the stationary phases, depletion of nutrient content forces it to shift towards a mode of survival which requires it to scavenge for nutrients exhibiting virulence factors and ROS protection. Interestingly, production of ROS is a common mechanism that bactericidal antibiotics induce cell death (Kohanski *et al.*, 2007). This means that with a rise in ROS metabolism proteins, the cells in the late stages of stationary phase are more resistant to the effects of antibiotics that deploy ROS to cause bacterial cell death. All this spell wonders as to the robust mechanism at *A. baumannii's* disposal to ensure its survival even at the harshest of conditions. It should be noted that these observations were made using reference ATCC strains isolated decades ago, which could suggest an even more robust mechanism for clinical strains.

There has been evidence to suggest that as a bacterial strain acquires the ability to resist antibiotics and becoming multi-drug resistant its biological fitness becomes affected negatively. These reports have been found in *A. baumannii*, *S. aureus*, *M. tuberculosis* and *E. faecium* (Ender *et al.*, 2004; Enne *et al.*, 2004; Mariam *et al.*, 2004). In a study (Fernández-Reyes *et al.*, 2009) recently conducted, where an antibiotic susceptible *A. baumannii* ATCC 19606 strain was induced to later have Colistin resistance was compared with each other from a proteomic angle. In the comparison, 35 proteins were found to be differentially expressed with most of them appearing to be downregulated in the colistin-resistance strain. Among the 35 proteins were those from the outer membrane, chaperones, protein biosynthesis factors and metabolic enzymes. The downregulation of these proteins suggests that the fitness of the bacteria is a trade-off that needed to be made in order for the bacteria to survive in an antibiotic environment. An example in the similar experiment can be seen with regards to metabolism where generation time of the colistin-resistant strain was 62 minutes as compared to the wild type which was 40 minutes. Protein synthesis was also affected as ribosomal proteins such as L15 known as a late-assembly component of the large ribosome subunit involved in the integration of 5S rRNA into the ribosome was also found to be downregulated in the resistant strain (Kaczanowska and Rydén-Aulin, 2007; Fernández-Reyes *et al.*, 2009). All this seemingly points to a reduction in overall fitness for the resistant strain and perhaps may warrant further studies to see its relevance in a Malaysian context.

Recent computational advances in the field of genomics have allowed for a comparative analyses of multiple *A. baumannii* clinical isolates. These advances have shown that *A. baumannii* possesses a remarkable capacity to acquire and rearrange its genetic makeup providing vital clues in uncovering the species population structure and phylogenetic relationships among strains specifically on the origin of antimicrobial resistance and virulence factors (McConnell *et al.*, 2013; Antunes *et al.*, 2014). As more strains are analysed, the whole-genome repertoire of *A. baumannii* expands greatly. Thankfully, with the data we can identify that comparatively the core genome of *A. baumannii* stands at 2200 CDSs (Coding DNA Sequences) which also corresponds to approximately 60% of the average strain genome content mainly related to general metabolic and cellular processes (Imperi *et al.*, 2011). Interestingly, the remaining 25-46% of the genes are unique to each strain's and make up of accessory genome that can encode for transport and transcription regulation functions, strain specific hypothetical proteins, transposases and insertion sequences (Adams *et al.*, 2008; Imperi *et al.*, 2011). These accessory genes are of great interest as their diverse variation could be a major contributing factor to the rapid onset of multiple MDR strains seen in the various outbreaks globally.

Taking a step back, when comparing the core genome to the genus *Acinetobacter*, the difference seems stark as it stands at a smaller 900-1300 CDS for 37 of the 38 *Acinetobacter* spp. which is 25-35% of *A. baumannii*'s CDSs (39). This difference begs the question to how *A. baumannii* could have varied so drastically from within its own non-pathogenic species within the genus. But among the other species in its genus, based on the sequencing of seven housekeeping genes, clinically relevant *A. baumannii* was found to be closely related genotypically to *A. calcoaceticus* and phenotypically difficult to distinguish (Diancourt *et al.*, 2010). Together with the clinically important *A. pittii* and *A. nosocomialis*, all 4 species form the *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex as phenotypically they are almost indistinguishable from each other (Fitzpatrick *et al.*, 2015). Their close core genome to each other demonstrate their ancestral lineage and that the evolution of *A. baumannii* to its current state was due to the expansion of its accessory genome. In addition, another study (Sahl *et al.*, 2013) demonstrated that very few potential virulence factors are exclusive to *A. baumannii* and that many of the putative virulence factors are conserved to the Acb complex. This evidence suggests that *A. baumannii* may have at some stage during its evolutionary history diverged from the Acb complex which could have been due to the direct or indirect introduction of intensive antibiotic therapies post-1950's, leading to the antimicrobial resistance gene acquisition mainly by horizontal transfer (Da Silva and Domingues, 2016). Compare this with ATCC 19606 strain which was isolated more than 5 decades ago and also antibiotic susceptible and also possesses much of the same core genome which houses several virulence factors that have been implicated in infections, suggesting that *A. baumannii* is pathogenic by nature but only made successful thanks to acquiring antimicrobial resistance. All this could explain the simple proteomic variation observe in this current study.

CONCLUSION

In summary, though significant advances have been made in understanding *A. baumannii* in the recent decades have shed much light on the success of the pathogen both in terms of genomic and proteomic data, the wider diversity of the *A. baumannii* population mainly

ISSN (print): 1823-8262, ISSN (online): 2231-7538

those in Malaysia have yet to properly characterized. Highlighting the simple variation that can be seen in a clinically relevant strain locally when compared to reference strains, comparative genomic studies should therefore be complimented by similar extensive proteomic analysis in order to expand the already large repertoire of clinical and nonclinical *A. baumannii* isolates within the country first, before attempting to compare these with results globally. It is also recommended that further protein identification be carried out as well to validate the existing results as we seek to gain further understanding of the processes leading to the evolution of this species as a global pathogen.

ACKNOWLEDGEMENTS

The authors would like to extend their gratitude to Taylor's University for providing the facilities and funding in the form of a generous grant under the Taylor's University Research Grant Scheme (TRGS/MFS/2/2014/SOM/005).

CONFLICT OF INTEREST

The authors of this study declare that there is no conflict of interests in relation to the publication of this manuscript.

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