Background
Antimicrobial resistance is one of the major threats to global health, resulting in an increasing number of people suffering from severe illness or dying due to infections that were once easily curable with antibiotics. *Pseudomonas aeruginosa* represents one of the most concerning pathogens involved in antibiotic resistance where the World Health Organisation has classified this gram-negative bacterium as an ESKAPE organism, and it is also listed in the Priority 1: Critical list. Hence, in this project, we opted for a novel intervention by using aptamers to inhibit the growth of *Pseudomonas aeruginosa,* which can be cost-effective and less laborious. Besides, antimicrobial agents targeting the outer membrane protein (OMP) in Gram-negative bacteria can also be effective at killing or inhibiting bacterial growth as these proteins play an important role in bacteria survival as well as being exposed to the surface, which facilitates direct binding. Aptamer repurposing also reduces the cost and length of new drug development.
Methodology
Unmodified functional DNA aptamers from the Aptagen database were docked with the essential OMP using various docking tools such as HADDOCK 2.4 and HDOCK web server to determine the binding site and binding score of the various aptamers. Aptamers that bind to the active site with a good binding score were synthesised and folded into their 3D structures. *P. aeruginosa* cells were incubated with the aptamers overnight and the inhibitory effects on the bacterial growth curve were investigated.
Results and Discussion
Apt31 had the best HADDOCK score, and was found to bind near the active site of the OMP. Interestingly, this aptamer also exhibited significant antibacterial activity from the early stationary growth phase onwards. The addition of the aptamer after the late log phase also exhibited a similar effect. Besides, the antibacterial activity of apt31 was also dose-dependent. Hence, we can deduce that apt31 binds to the active site region and blocks the OMP activity. The major role of this OMP is to fold and insert β-barrel proteins into the outer membrane layer. As apt31 hinders the OMP activity, we postulate that the bacteria cells eventually die due to a lack of OMPs, which are essential for survival and virulence.
Conclusion
We show that the apt31, an aptamer that binds to an antitumor, is capable of binding to OMP in *P.aeruginosa.* The aptamer-OMP complex exhibits a significant antibacterial effect in a dose-dependent manner. Future experiments will include the expression of the recombinant OMP and determination of the dissociation constant (K_d) of the aptamer-OMP complex.

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Production of recombinant shark V_{NAR} single domain antibody specific against DENV Type 2 NS1

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Background
Dengue virus (DENV)) infection is one of the main global health issues leading to high morbidity and death rate. The accuracy of early diagnosis is the key for effective treatment. However, early diagnosis for DENV infection is a great challenge due to the similar onset symptoms of Dengue with other flaviviruses, concurrent infection and cross-reactivity among dengue serotypes and other flaviviruses [1]. Besides, the limitations of conventional monoclonal antibodies (mAbs) also affect the efficiency of diagnostic kits [2]. Alternatively, the variable new antigen receptor (V_{NAR}) found in shark, has recently been identified as a promising diagnostic tool due to its excellent thermostability and capability to target on cryptic epitopes [3]. In this study, a potential clone that recognizing DENV Type 2 NS1 has been isolated and expressed. This recombinant protein was determined to be soluble and potentially developed as a novel reagent for DENV immunodiagnostic platform.
Methodology
A semi-synthetic library of shark V_{NAR} was used for antibody selection. Total of 3 rounds of biopanning was undertaken in respect to isolate potential binders which can specifically recognize DENV Type 2 NS1. The eluted phage from each round of biopanning were subject to polyclonal and monoclonal phage ELISA. After verified through DNA sequencing, the selected clone was expressed using bacteria expression system, followed by immobilized metal affinity chromatography (IMAC) purification process. The biological functions of recombinant V_{NAR} will then be characterized, including thermostability, specificity and sensitivity towards DENV Type 2 NS1.
Results and Discussion
For monoclonal phage ELISA, G3, Z3 and Z8 clones were identified to possess good affinity towards NS1 and lower cross reaction towards BSA. Thus, Z8 clone was selected to express as a 12 kDa protein with some optimization during protein expression. To verify target protein, the recombinant antibody reacted with anti-His6 antibody was performed using Western Blot. The functional assays of recombinant protein Z8 are currently underway.
Conclusion
Z8 clone isolated from biopanning has been identified as a potent binder. The recombinant anti-NS1 V_{NAR} Z8 antibody was successfully produced in a bacteria expression system. This new binder can be developed as new reagent for DENV immunodiagnostic platform.

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P-37

A fatal case of cerebral malaria complicated with *Gemella bergeri* bacteremia: Role of *Plasmodium* mitochondrial *cox3* gene and MALDI-TOF Mass Spectrometry for species identification

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Background
Here, we described a fatal case of a 37-year-old returning traveller from Burkina Faso, West Africa, who presented with an acute fitting episode later diagnosed as cerebral malaria induced by *Plasmodium falciparum* based on microscopic examination and *Plasmodium* mitochondrial cytochrome c oxidase III (*cox3*) gene PCR target. Unfortunately, the patient passed away due to severe malaria with multiorgan failure complicated with *Gemella bergeri* bacteremia. *G. bergeri* was identified using MALDI-TOF mass spectrometry.
Methodology
Preliminary evaluation of blood film malaria parasite showed coinfection of *P. falciparum* and *Plasmodium malariae*. Further PCR-based detection analysis using the *Plasmodium* mitochondrial *cox3* gene was used to identify the malaria species. The patient's blood culture revealed gram-positive cocci in clusters, and further identification using MALDI-TOF Mass Spectrometry was done. Informed consent to publish had been obtained.
Results and Discussion
The patient blood sample was positive for *P. falciparum* based on analysis using a PCR assay targeting the *cox3* gene. Further, analysis done at Makmal Kesihatan Awam Kebangsaan confirmed *P. falciparum* infection. His blood culture revealed gram- shown in our case, cerebral malaria induced by *P. falciparum* causes coma, long-term positive cocci in clusters, which was later identified as *G. bergeri* using MALDI-TOF mass spectrometry. Despite the initiation of intravenous artesunate and subsequent parasite count responding and showing parasite clearance, the complication is detrimental. As neurological consequences and death. The patient also had concomitant fatal *G. bergeri* bacteremia. Initial examination revealed that the patient had poor oral hygiene and a missing tooth which could be the source of *Gemella* infection, which is common in the oral cavity and a causative organism of septic shock.
Conclusion
This report is the first fatal case of cerebral malaria caused by *P. falciparum* coinfection with *G. bergeri* bacteremia. Therefore, it is critical to determine the exact aetiology for appropriate medical management. *Plasmodium* mitochondrial *cox3* gene and MALDI-TOF Mass Spectrometry are useful for species identification, as illustrated in this case.

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Association of *MTHFR* polymorphism in stroke risk and rehabilitation outcomes

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Background
There are 12.2 million new stroke cases annually and about 101 million people worldwide are living with stroke aftermath [1]. In Malaysia, stroke is the third leading cause of death. The rs1801133 single nucleotide polymorphism (SNP) in the methylenetetrahydrofolate reductase (*MTHFR*) gene has been linked to stroke pathogenesis in a recent meta-analysis [2]. To date, there is a lack of study addressing this SNP towards stroke risk and rehabilitation outcomes in the Malaysian population. Hence, this study aims to investigate the association of *MTHFR* rs1801133 SNP with the risk and rehabilitation outcomes in Malaysian stroke patients.
Methodology
The peripheral blood sample was collected from 251 age-matched individuals (113 stroke patients and 138 healthy controls without a stroke history) with written consent. The genomic DNA was extracted from these blood samples and the rs1801133 SNP was genotyped using a TaqMan® assay approach. The odds ratio and 95% confidence interval were calculated for risk association analysis. The stroke patients were subjected to a special-designed rehabilitation exercise and their pre- and post-exercise assessments including the Barthel index, Fugl-Meyer assessment-upper extremity, and Fugl-Meyer assessment-lower extremity were compared based on their genotypes.
Results and Discussion
This study showed that the presence of a T allele of the rs1801133 SNP had at least a 0.4-fold reduced risk of stroke. This suggests that the T allele of the rs1801133 SNP is protective against a stroke in the Malaysian population. Surprisingly, the finding of this study is in contrast with those reported in the meta-analyses [2,3]. Out of the 31 stroke patients who had completed their special-designed rehabilitation exercises, patients with a homozygous (C/C) genotype showed significant improvement in the post-rehabilitation assessments including the Barthel index, Fugl-Meyer assessment-upper extremity, and Fugl-Meyer assessment-lower extremity.
Conclusion
In conclusion, this study suggests that the *MTHFR* rs1801133 SNP is a potential biomarker for stroke risk and rehabilitation outcome predictions in Malaysian stroke patients. Data of this study could be useful for stroke management in the country.

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P-39

RNU6B and miR-16 as stable normalisation control for relative RT-PCR of Urinary microRNA in patients with colorectal polyps

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Background
Relative real-time polymerase chain reaction (RT-PCR) is an experimental technique widely used in quantifying the expression of a particular gene of interest. However, the reliability of gene expression data obtained through this technique is highly dependent on the stability of the housekeeping genes being used. Housekeeping genes act as a normaliser or endogenous reference in the relative