COMPARATIVE PROTEOMIC ANALYSIS OF CLINICAL AND ENVIRONMENTAL ISOLATES OF *Aeromonas dhakensis* USING TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Aeromonas dhakensis is a ubiquitous Gram-negative rod-shaped organism that is commonly isolated from aquatic environments and has now emerged as an important human pathogen. It causes a broad range of intestinal and extra-intestinal diseases such as gastroenteritis, skin and soft tissue infections and septicemia in both immunocompromised and immunocompetent individuals. Moreover, *A. dhakensis* was also reported to be the most virulent species among all the *Aeromonas* spp. with higher toxicity, higher biofilm formation and minimum inhibitory concentration. Recently this species had caused two fatal cases of bacteremia and necrotizing fasciitis in patients co-infected with severe dengue infection. Yet, information on *A. dhakensis* virulence factor is still limited. Hence, proteome profiles of *A. dhakensis* was investigated and differentially expressed proteins were identified in clinical strains compared to the environmental strains during exponential and stationary growth phases. A total of 6 previously isolated and molecularly characterised *A. dhakensis* strains (clinical, n=3; environmental, n=3) were included in this study. Bacterial cultures were first harvested at exponential (2.5 hours) and stationary (6 hours) growth phases, followed by extraction using mechanical disruption method, quantified using 2D-Quant Kit and then cleaned-up with 2D Clean-Up kit. Protein samples were separated using 2D-PAGE (pH range 4-7). Gel analysis was performed using ImageMaster 2D Platinum and spots of interest were identified using MALDI-TOF. Each silver-stained gel contained an average of at least 200 protein spots. A total of 9 protein-spots were detected from 7 gels in the clinical strains only, in which 4 and 5 spots were present in stationary and exponential phase cultures, respectively. These proteins were excised, destained and sent for identification using MALDI-ToF. It is speculated that the 9 differentially expressed protein spots may contribute to the virulence of *A. dhakensis*. However, their functions and mechanisms are yet to be determined.

Keywords: *Aeromonas dhakensis*, Exponential, Proteomic, Stationary, Virulence factor

AVIAN INFLUENZA (AIV) A H5N1 VIRUS-INDUCED APOPTOSIS IN MDCK AND VERO CELL LINES

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Avian influenza virus (AIV) A H5N1 is a major concern of a pandemic flu outbreak in the future. Previous studies showed the involvement of apoptosis in H5N1 pathogenesis. Therefore, understanding H5N1-induced apoptosis could facilitate development of antivirals targeting H5N1-associated apoptosis. This study was conducted to determine the expression of pro-apoptotic and anti-apoptotic genes in H5N1-infected MDCK and Vero cell lines. MDCK and Vero cell lines at 80% confluency were infected with H5N1 virus at $10^{2.5}$TCID50/ml infectious dose. Infection was allowed for 8, 24, 48, 72, 96 and 120 hours. Cells were harvested at designated time points and the total RNA was isolated and then reverse-transcribed into cDNA. Pro-apoptotic (Bax, Fas-L, TRAIL, caspase 3 and caspase 8) and anti-apoptotic genes (Bcl-2) were amplified by polymerase chain reaction (PCR) using specific primers. The amplified products were separated on 1% (w/v) agarose gel to determine the gene expression. Significant cell death was observed in both H5N1-infected MDCK and Vero cell lines especially at 96 hours post-infection (hpi). Comparing both infected cell lines, MDCK cells were more susceptible to H5N1 infection than Vero cells. DNA fragmentation was observed in H5N1-infected MDCK and Vero cell lines at 72 hpi. Expressions of bax, Fas-L, TRAIL, caspase-3 and caspase-8 genes were downregulated at 8-48 hpi but were upregulated at 72-120 hpi. On the contrary, the anti-apoptotic gene, Bcl-2 was upregulated at 8-48 hpi and then downregulated at 72-120 hpi in both cell lines. As a conclusion, H5N1 could regulate apoptosis in both MDCK and Vero cell lines. Thus, H5N1-induced apoptotic pathway could be a potential target for future anti-influenza development.

Keywords: H5N1, MDCK, Apoptosis

ANTIBACTERIAL ACTIVITY OF *Centella asiatica* AGAINST *Mycobacterium smegmatis*

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Tuberculosis (TB) is an air borne disease which becomes one of the top 10 causes of death around the world. Although its percentage of incidence decreases by each year, the number of cases reported are still worrying. Due to that, researchers have worked on to find alternative treatment for TB including using natural plants in order to reduce the number of cases and to improve the limitations possess by synthetic drugs. *C. asiatica*, which have believed to have many benefits, is listed as one of the possible alternatives in treating TB. However, research in finding alternative drug against *M. tuberculosis* cannot be performed directly because it requires high level facilities such as BSC level 3 since *M. tuberculosis* is an airborne pathogen which easily infects human. Therefore, less virulent mycobacterium such as *M. smegmatis* have been selected to replace *M. tuberculosis* because they have many similarities, including drug reactions and antibacterial activity. This study was conducted to evaluate the ability of *C. asiatica* as an antibacterial agent against *M. tuberculosis* by using *M. smegmatis* as a substitute organism. In this study, the antibacterial activity of *C. asiatica* against *M. smegmatis* was studied through two aspects, i.e. morphological changes based on staining, shape and length, and anti-bacterial activity tested by disk diffusion method, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The results showed that *C. asiatica* had an effect on the *M. smegmatis* morphology. However, this plant extract was unable to inhibit the growth of the mycobacterium either through disk diffusion method, MIC or MBC. In conclusion, the findings showed that *C. asiatica* extract was unable to inhibit *M. smegmatis* growth directly, but had an indirect effect on the organism by changing its morphology.

Keywords: *Centella asiatica, Mycobacterium smegmatis*

THE INVOLVEMENT OF PNEUMOCOCCAL HYALURONIDASE IN PNEUMOCOCCAL BIOFILMS FORMATION AND DETACHMENT

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Streptococcus pneumoniae causes many severe infections and mortality cases due to its ability to form biofilms. Antimicrobial resistance and difficulties of host immune system to combat the biofilms make the treatment against *S. pneumoniae* become harder. Thus, investigations on pneumococcal biofilm are significantly important and needed. Pneumococcal biofilm was found to build up and enhanced by various molecules and one of them is hyaluronic acid (HA). Previous *in vivo* and *in vitro* studies found that HA served as substrate for hyaluronidase (Hyl) enzyme. This enzyme is produced by various organism including *S. pneumoniae*. However, the correlation between this enzyme-substrate reaction directly towards biofilms activity (formation and detachment) is not well discussed. Thus, this fundamental study was conducted to investigate the role of pneumococcal Hyl on *S. pneumoniae in vitro* biofilms formation and detachment. Wild type and Hyl mutant strain (ΔHyl-) of *S. pneumoniae* were grown in brain heart infusion (BHI) media supplemented with and without HA and glucose for 96 hours. The formed biofilms in each interval incubation time were detected by using crystal-violet (CV) microtiter plate assay. Formation of biofilms was highly produced in glucose-supplemented BHI media compared to HA-supplemented BHI media, regardless of type of the strain. Comparison between the strains showed wild type strain produce higher biofilms formation than ΔHyl- strain in both media (glucose- and HA-supplemented BHI media). Detachment of biofilms was detected in all groups within 96 hours of incubation, where the least detachment was observed in wild type strain in BHI media without any supplementation. The results of this study suggested that the pneumococcal Hyl may involve in the formation and detachment of the pneumococcal biofilms. This finding can be used to plan alternative strategies to encounter biofilms-based infections in *S. pneumoniae* in future.

Keywords: *Streptococcus pneumoniae.* biofilm

MOLECULAR TYPING OF METHICILLIN-RESISTANCE *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATES FROM A STUDENT POPULATIONS

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Methicillin-resistance *Staphylococcus aureus* (MRSA) infections is one of the most reported multidrug-resistant infections in healthcare setting worldwide. Although MRSA were rarely found in healthy person, but they were not excluded from exposed to this subsequent infections that transmitted by the MRSA carrier. This study is carried out to determine nasal carriage prevalence of *S. aureus* isolated from student populations and to identify the genetic variability of MRSA strain. Nasal carriage samples were obtained from 166 students age between 18-25 years old in Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The self-collected nasal samples were cultured on Mannitol Salt Agar (MSA) and incubated at 37^{o}C overnight. The *S. aureus* isolates were confirmed based on the colour changes on MSA. All *S. aureus* isolates were tested for antimicrobial susceptibility by using Kirby-Bauer disc-diffusion method. Polymerase chain reaction (PCR) was carried out to screen for MRSA by the detection of *mecA* gene (533bp). The *mecA*-positive isolates were subjected to random amplification of polymorphic DNA (RAPD) analysis using 1254 primer. A dendogram was constructed using the RAPD data generated by PyElph 1.4 program. Out of 166 samples, 50 of the nasal swabs grown on the MSA plates were positive with *S. aureus* and six isolates (3.61%) were positive for *mecA* gene. Furthermore, antimicrobial susceptibility test showed that the highest frequency of resistance was observed for penicillin 82 %(41), followed by cefoxitin 26%(13). There were low resistance rate observed in tetracycline, erythromycin and gentamicin (<14%). Based on the dendogram analysis, the MRSA isolates produced two distinct but related RAPD clusters which were well distinguished. A moderate rate of *S. aureus* carriage and low frequencies of MRSA were detected in healthy students. The data from this study can be use to determine the risk factors contributed to MRSA colonization in carrier.

Keywords: *Staphylococcus aureus*, MRSA, RAPD PCR

CROSS REACTIVITY BETWEEN SIKAMAT VIRUS (SIKV) AGAINST NELSON BAY VIRUS (NBV) AND SEROTYPE 3 MAMMALIAN ORTHOREOVIRUS (MRV3)

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Pteropine orthoreovirus (PRV) is a newly emerging respiratory virus that is circulating among the regions of Southeast Asia. Four PRV strains were isolated from Malaysia and this research aimed to generate polyclonal antibodies against one of the PRV strains which is known as the Sikamat Virus (SikV) and to study the cross reactivity among different strains of PRV, specifically SikV and Nelson Bay Virus (NBV), and another closely related species of virus which is the Mammalian orthoreovirus (MRV3) to understand the antigenic relationship within these species group. Polyclonal antibodies against SikV were generated through immunisation of rabbits. The dot blot assay was then performed by reacting the anti-SikV antibodies against SikV to detect the presence of antibodies in the collected sera. Another dot blot assay was conducted to test for presence of cross reactivity between anti-NBV and anti-MRV3 antibodies against SikV. The presence of dark spots from dot blot assay indicated binding of antibodies with viral antigen. This indicates the presence of antibodies against SikV in the sera after immunisation. Presence of cross reactivity of anti-SikV antibody and anti-NBV antibody against SikV can be observed while there is no cross reactivity between anti-MRV3 antibody and SikV. The results suggest that the generated antibodies against PRV strains can cross react among each other but not with another species. This study is the first to test the cross reactivity of anti-NBV antibody and anti-MRV3 antibody against SikV. Viral micro-neutralisation assay is expected to be done as a confirmatory test to determine the neutralisation titre of SikV. PRV strains are capable of cross reacting with one another but not with another species of virus.

Keywords: Pteropine orthoreovirus, serum neutralisation, serology

CHARACTERIZATION AND ANTIBIOTIC RESISTANCE PROFILE OF *Salmonella* ISOLATES OBTAINED FROM STREED VENDED BEVERAGES SOLD IN CHOW KIT

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Salmonella enterica subsp. *enterica* poses threat toward both animal and human. *Salmonella* infection are the primary cause of food poisoning in many countries for over 100 years. There were more than 2600 serotypes of *Salmonella* have been reported to date. The current study was to find out the serotypes of *Salmonella* present in street vended beverages obtained from Chow Kit, their antibiotic resistance profile against selected antibiotics and the antibiotic resistance genes present in the *Salmonella spp*. There were 19 isolates of *Salmonella* obtained from the street vended beverages including cordial drinks, milk-based drinks and fruit juices. The isolates were streaked on Xylose Lysine Deoxycholate (XLD) agar and black colonies were present which can be identified as *Salmonella spp*. The isolates were serotyped by using 16s rRNA Polymerase Chain Reaction (PCR) method and then were screened for antibiotic resistant profile by using Kirby-Bauer disc diffusion method. The antibiotic resistance genes for the selected isolates were investigated by using PCR method. Among 19 isolates of *Salmonella spp.* obtained, 100% were resistance to antibiotic Erythromycin (E15) and Penicillin (P10), 42%, 21%, and 10% towards Tetracycline (TE30), Cephalexin (CL30) and Ampicillin (AMP10), respectively. The zone of inhibition for all isolates tested with Erythromycin and Penicillin are 0 mm, 8 mm for Tetracycline in average, 13 mm for Cephalexin and 12 mm for Ampicillin. The presence study showed that street vended beverages sold in Chow Kit were contaminated with *Salmonella spp.* in which have relatively high resistance toward antibiotics. It could pose public health and therapeutic problems in consumers as potential vehicle of resistant *Salmonella* foodborne infection.

Keywords: *Salmonella*, antibiotic, PCR

MICROBIOLOGICAL QUALITY OF SUSHI SAMPLED FROM SELECTED FOOD OUTLTES IN KOTA BHARU, KELANTAN

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Sushi is one of the Japanese food that gaining popularity among Malaysian. However, as a ready-to-eat food sushi which needs minimum preparation is prone to microbial contamination and it has been associated with foodborne outbreaks. This study was carried out to evaluate the microbiological quality of sushi sampled from 3 different food outlets in Kota Bharu, Kelantan from July 2018 until August 2018. A total of thirty samples (n=30) were analysed via total plate count (TPC), total coliform count (TCC), faecal coliform count (FCC) and presence of *E. coli*. Unsatisfactory results which exceeded the proposed acceptable guideline for ready-to-eat food were seen in a total of 25 (83.3%) samples for total plate count, 10 (33.3%) samples for total coliform count and 7 (23.3%) samples for faecal coliform count. There was no *E. coli* detected in all 30 sushi samples. As a conclusion, sushi samples from selected food outlets in Kota Bharu, Kelantan were unsatisfactory in terms of hygiene and its safety is doubtful. Appropriate preventive measures and hygienic practices should be followed to ensure the safety and quality of sushi.

Keywords: Sushi, Microbiological quality, *E. coli*

IN VITRO ANTIVIRAL ACTIVITY OF *Ganoderma neo-japonicum* MUSHROOM EXTRACTS AGAINST ENTEROVIRUSES THAT CAUSE HAND, FOOT AND MOUTH DISEASE

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Hand, foot and mouth disease (HFMD) is a highly contagious pediatric disease that mainly affects infant and children younger than 5 years old. HFMD is commonly caused by enterovirus A71 (EV-A71) and coxsackievirus A16 (CV-A16). The lack of effective therapies and/or multivalent vaccines against enteroviruses that cause HFMD have urged a growing need for developing new, effective, and broad-spectrum antiviral agents to prevent and/or ameliorate the disease. Thus, the objectives of this study are: 1) To investigate the antiviral activity of *Ganoderma neo-japonicum* mushroom extracts against enteroviruses that cause HFMD; 2) To determine the antiviral mechanism(s) of the selected mushroom extract. In this study, we have tested 4 mushroom extracts (S1-S4) to evaluate their abilities to inhibit EV-A71, CV-A16, and CV-A10 infection and replication on primary human oral fibroblast cells. MTS assay was performed to determine the maximal non-toxic dose (MNTD) of the extracts, before they were screened for antiviral activity. The antiviral effect of the extracts on EV-A71 and CV-A16 replication was determined using cytopathic inhibition, immunocytochemistry (to detect viral antigens) and cell culture infectious dose 50 assays. Mushroom extracts that demonstrated strongest antiviral effects against EV-A71, CV-A16 or CV-A10 were selected for further evaluation. Mushroom extract (S2) (MNTD range 1.25-0.078 mg/ml) demonstrated highest antiviral activity against EV-A71 and CV-A16, but not CV-A10. S2 extract caused direct virus inactivation and preventing virus from efficiently replicating in cells. However, S2 failed to inhibit virus attachment and/or entry into pre-treated cells. Taken together, this study revealed that *G. neo-japonicum* extract (S2) can inhibit EV-A71 and CV-A16 infection and replication with a dual mode of action. In conclusion, *Ganoderma neo-japonicum* extract (S2) is potential to be further developed into an effective and safe anti-enteroviruses agent.

Keywords: Hand, Foot and mouth disease, *Ganoderma neo-japonicum*, Antiviral

ISOLATION OF MICROBES FROM COCOA SAMPLES: COMPARISON BETWEEN ENVIROMENT AND DURING FERMENTATION PROCESS FOR THEIR BIOACTIVITY

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The knowledge on cocoa fermented microbes is still infancy as there is still no reported study was carried out from different clones of Malaysia cocoa plants. Therefore, it is an opportunity to find new microbes with potential producing bioactive compounds. In the cocoa fermentation process, microbes that help in fermentation mainly are yeast, lactic acid and acetic acid bacteria. The aim of this study was to isolate the microbes from the environment of cocoa fermentation and during the cocoa fermentation at different layers of the fermentation and to determine the macromorphology of the isolates and its bioactivity against ESKAPE human pathogens. In this study, 18 kg of two different cocoa bean clones which were BR25 and a mixture of BR25 and KKM22 were fermented for 120 hours and samples were taken every 12 hours. The plating was done on MRS agar for lactic acid bacteria, PCA for any growth of bacteria, modified GYC agar for acetic acid bacteria and YEA agar for yeast. The antimicrobial activity against ESKAPE human pathogen was carried out by using a well agar diffusion method for lactic acid bacteria while spread and patch method on MHA agar for acetic acid bacteria and yeast. From BR25 clone, there were 39 isolates from GYC agar, 16 isolates from PCA agar and 40 isolates from MRS agar while for MIX clones, 20 isolates from GYC agar, 36 isolates from PCA and 78 isolates from MRS agar. For antimicrobial activity, isolation of microbes from cocoa samples showed no inhibition zone against ESKAPE human pathogens. In conclusion, the results showed that the bioactivity of the isolates from the environment and during the fermentation process did not show any reactions against ESKAPE human pathogens.

Keywords: Cocoa bean fermentation, ESKAPE human pathogen, Antimicrobial activity, Lactic Acid Bacteria, Acetic Acid Bacteria, Yeast, *Theobroma Cocoa.*

THE INFLUENCE OF ACCOMPANYING BACTERIA ON GROWTH OF *BLASTOCYSTIS* SP. SUBTYPE 3

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Blastocystis sp. is an intestinal parasite found in humans with symptoms including abdominal discomfort, diarrhea and flatulence. However, its pathogenicity is usually queried as the parasite as it is also present in asymptomatic individuals. At least seventeen subtypes are recognized within *Blastocystis* sp. based on molecular analysis of small subunit ribosomal RNA (SSU-rDNA) gene, with Subtype 3 (ST3) as the most prevalent subtype among humans. This subtype has been shown to be associated with irritable bowel syndrome (IBS), Crohn's disease and colorectal cancer. Morphological and metagenomics studies have revealed the presence of accompanying bacteria in the *in vitro* culture. Axenization of this parasite is always a challenge as its growth is suppressed in the absence of accompanying bacteria. This study aimed to identify the influence of accompanying bacteria towards the growth of *Blastocystis* sp. ST3. The experiment performed by exposing the parasites with meropenem (against Gram-negative bacteria), vancomycin (against Gram-positive bacteria) and augmentin (broad spectrum) at 4 different concentrations *in vitro*. Parasite count was performed regularly for six to seven days. Simultaneously, bacteria-containing supernatant of treated and control cultures were cultured on various types of agar. The colonies grown were identified by polymerase chain reaction (PCR). Morphological changes of antibiotic-treated ST3 have also been studied. At the highest concentration of meropenem (8µg/ml) and augmentin (32µg/ml), the number of symptomatic *Blastocystis* sp. ST3 was 1- to 2-fold declined compared to control. For asymptomatic isolate, the number of cells was 7-fold increased at highest concentration of meropenem (8µg/ml) but 2-fold decreased at the highest concentration of vancomycin (16µg/ml). This is the first study to show that the bacteria do play a role towards the growth of *Blastocystis* sp. ST3, revealing the symptomatic isolate has a symbiotic relationship with both Gram-positive and Gram-negative strains that could be of resistant (pathogenic) strains.

Keywords: *Blastocystis* sp. ST3, accompanying bacteria, growth

DENGUE VIRUS TYPE-2-INDUCED APOPTOSIS IN MDCK AND VERO CELL LINES

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Dengue is one of the most important arthropod-borne human diseases. Previous studies showed different host permissiveness and susceptibility to dengue virus (DENV). Permissive host cells undergo apoptosis in response to DENV infections. In this study, expressions of apoptotic genes in DENV-2-infected MDCK and Vero cell lines were investigated. Both cell lines were grown in RPMI-1640 supplemented with 10% FBS and 1% Pen-Strep. DENV infection was performed with MOI of 0.1 pfu/cells and infected cells were harvested at 12, 24, 48, 72, 96, 120, 144, 168 and 192 hours post-infection (hpi). To determine the expressions of apoptotic genes (TRAIL, Caspase-3, p53 and Bax), the total RNA was extracted from infected cells and then converted into cDNA. The apoptotic genes were amplified using specific primers and cDNA. DNA fragmentation in DENV-infected cell lines was determined using agarose gel electrophoresis. DENV-2 infection in MDCK and Vero cell lines with DENV2 triggered cell death and apoptosis. Vero cells were more susceptible to DENV infection compared to MDCK cells especially at 72-168 hpi. Analysis of DNA fragmentation which is a hallmark of apoptosis was more obvious in DENV-infected Vero cell line at 72 hpi than infected MDCK cell line. TRAIL, Caspase-3, p53 and Bax gene expressions were downregulated at 12-48 hpi but upregulated at 72-168 hpi in DENV-infected Vero cells. However, DENV-infected MDCK cells only showed upregulation of apoptotic gene expressions at 120 hpi. Conclusively, DENV-2 infection regulates apoptotic gene expressions in MDCK and Vero cell lines hence promotion of cell death. By understanding how DENV-2 utilizes host's apoptotic mechanism to its advantages, it is possible to develop alternative antiviral drugs for dengue infection that interfere with virus survival.

Keywords: Dengue Virus, *Flavivirus*, apoptosis

IDENTIFICATION AND INVESTIGATION OF *Elizabethkingia* AND *Chryseobacterium* SPECIES USING 16S RIBOSOMAL RNA GENE SEQUENCING AND ANTIBIOTIC SUSCEPTIBILITY TESTING

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Members of *Flavobacterium, Elizabethkingia* and *Chryseobacterium* are saprophytes but recently emerged as opportunistic human pathogens. They cause infections ranging from sepsis, bacteraemia, pneumonia to meningitis particularly in infants and immuno-compromised patients. The ability of these organisms survive in the hospital environment is suggested linked to antibiotic resistance and their capacity to form biofilms. They are able to cause life-threatening infections which required empiric antimicrobial therapy, thus, accurate species identification and the antibiotic susceptibility profiles of these organisms warrant an investigation The aim of this study is to identify and speciate *Flavobacterium* using 16S rRNA gene sequencing and further investigate their antimicrobial susceptibility profiles. A total of 57 archived clinical isolates of *Flavobacterium* obtained from University Malaya Medical Center were included in this study. Bacterial genomic DNA was extracted and genetically reconfirmed using 16S rRNA gene with primers, 27F: 5'-AGA GTT TGA TCM TGG CTC AG-3' and 1492R: 5'-GGY TAC CTT GTT ACG ACT T-3'. Phylogenetic trees were constructed based on Neighbor-Joining method via MEGA 7 program with bootstrapping for 1000 replicates. The susceptibility of these isolates to 15 antimicrobial agents were examined using Kirby Bauer disk diffusion method and interpretation of inhibition zone was carried out according to CLSI guidelines. Three species of *Elizabethkingia* and seven species of *Chryseobacterium* were identified: *E. anophelis* (n=16), *E. endophytica* (n=9), *E. miricola* (n=6); *C. gleum* (n=6), *C. flavum* (n=6), *C. bernardetii* (n=3), *C. gallinarum* (n=6), *C. cucumeris* (n=2), *C. rhizoplanae* (n=2), and *C. indologenes* (n=1). Resistance to imipenem (100%, 80.8%) and gentamicin (90.3%, 50.0%) were most commonly observed for both *Elizabethkingia* and *Chryseobacterium.* Multidrug-resistant patterns were observed in 29% (9/31) of the *Elizabethkingia* strains and 42.3% (11/26) in *Chryseobacterium* strains.In conclusion, 16S rRNA gene sequencing enable differentiation among *Flavobacterium* at species level and 18 multi-drug resistant strains were detected.

Keywords*:* 16S rRNA gene sequencing, antibiotic susceptibility, *Chryseobacterium, Elizabethkingia, Flavobacterium*

THE EFFECTIVENESS OF CONTACT LENS DISINFECTANT SOLUTIONS AGAINST *Acanthamoeba spp.* CYSTS OF CLINICAL AND ENVIRONMENTAL ISOLATES

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The usage of contact lens among the community is increasing due to trend and as an alternative of wearing glasses. However, most ocular diseases and eye infections as Acanthamoeba Keratitis are more related to contact lens usage. The contact lens disinfectant solutions are supposed to be able to eradicate the microorganisms that could possibly cause eye infections. Thus, study is made to see the effectiveness of the contact lens disinfectant solutions against the *Acanthamoeba sp.* cysts of clinical and environmental isolates. Disinfectant solutions from different brands with different contents are tested against four strains of *Acanthamoeba sp* in durations recommended by the product manufacturer; 4 and 6 hours and also in extended duration of 8 hours soaking. The disinfectant solutions used are Aosept plus and Biotrue multipurpose solution against the clinical strain (HKL55, HS19) and environmental strain (OF7, OT7[1]). The *Acanthamoeba sp* strains are subcultured onto non-nutrient agar and the cysts produced are mixed with disinfectants separately according to different soaking hours. After the soaking duration, the cysts are later cultured into non-nutrient agar layered with heat-killed *E.coli* and incubated in 30°C incubator. The cultures are observed under the inverted microscope for 14 days continuously to see any excystment of the cyst. The failure of the cysts to excyst indicates the efficiency of the disinfectant to kill the cyst successfully. Aosept plus was the only contact lens disinfectant solution able to inhibit the excystment of Acanthamoeba cyst in all four strains. Aosept plus solution provides assurance of safety from the Acanthamoeba Keratitis if used hygienically.

Keywords: Acanthamoeba, contact lens, disinfectants

CHARACTERIZATION OF A MOUSE MONOCLONAL IgM ANTIBODY AGAINST ENTEROVIRUS A71

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Enterovirus A71 (EV-A71) is a non-enveloped RNA virus classified under *Enterovirus* genus within the *Picornaviridae* family. EV-A71 is one of the major causative agent of Hand-Foot-and-Mouth Disease (HFMD). HFMD is usually self-limiting, but children infected with EV-A71 may developed severe and/or fatal cardiopulmonary and neurological complications. Previously, we have developed a mouse monoclonal antibody against EV-A71. However, the neutralization mechanism and epitope of this antibody remains unknown. Thus, the objectives of this study were 1) to determine the neutralizing mechanism of this monoclonal antibody against EV-A71 and 2) to identify neutralization epitope of EV-A71. Neutralizing mechanism was examined using pre- and post-attachment assays by pre-incubating EV-A71 with the MAb and treating the virus-bound cells, respectively. Antibody escape mutant was generated to map the location of the neutralizing epitope. After confirming the Ab escaped phenotype, viral RNA was extracted from the mutant and parental strains and followed by RT-PCR to amplify the EV-A71 P1 region before DNA sequencing. DNA sequences of the mutant were aligned with the parental virus to identify the amino acid change that may confer antibody resistance phenotype. The MAb could protect primary human oral fibroblast from EV-A71 infection in both pre- and post-attachment stage. A single amino acid substitution from lysine to glutamic acid at amino acid position 215 of VP1 was found in the mutant. This position 215 was located within the most exposed region (208-222) at the GH loop of VP1 and close to the canyon region. Thus, this MAb may block EV-A71 from binding to SCARB2 receptor, the main entry receptor of EV-A71. As a conclusion, this MAb could be further developed as a therapeutic agent against severe EV-A71-associated HFMD.

Keywords: Neutralizing monoclonal antibody, neutralizing epitope, Enterovirus A71

COMPARISON OF THE BINDING LEVEL OF DIFFERENT HUMAN ERYTHROCYTE DUFFY (FY) ANTIGENS TO *PLASMODIUM KNOWLESI* DUFFY BINDING PROTEIN ALPHA REGION II (PkDBPαII)

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Plasmodium knowlesi is the most common cause of human malaria in Malaysia. The invasion of its merozoite into human erythrocytes involves complex molecular interaction between *P. knowlesi* Duffy binding protein alpha region II (PkDBPαII) and Duffy antigen receptor for chemokines (DARC) on the erythrocytes. There are two immunologically distinct Duffy antigens, Fy^a and Fy^b. Studies showed that Fy^a has a significantly lower binding activity to *Plasmodium vivax* DBP compared to Fy^b, which is associated with a reduced risk of vivax malaria. However, information is scarce on the binding level of Fy antigens to PkDBPαII. The objective of this study is to measure and compare the binding level of Fy(a+b-) and Fy(a+b+) human erythrocytes to PkDBPαII. Fy(a+b-) and Fy(a+b+) healthy blood donors were recruited and genotyped (n=4 for each type). PkDBPαII of both Peninsular Malaysia and Malaysian Borneo haplotypes were expressed using COS-7 mammalian cells and incubated with the Fy(a+b-) and Fy(a+b+) erythrocytes. The binding level of erythrocytes to PkDBPαII was determined by counting the rosettes formed in erythrocyte-binding assay, which positive rosette is defined as adherent erythrocytes covering more than 50% of the surface of COS-7 cell. All erythrocyte-binding assays were performed in duplicates. For Peninsular Malaysia haplotype, the number of rosettes formed with Fy(a+b-) and Fy(a+b+) erythrocytes were 27.00 ± 4.97 and 65.00 ± 17.32, respectively (p=0.006). For Malaysian Borneo haplotype, 3.50 ± 1.91 and 26.00 ± 10.42 rosettes were obtained for Fy(a+b-) and Fy(a+b+) erythrocytes, respectively (p=0.021). No rosettes were observed in negative control. As a conclusion, Fy(a+b+) displayed a significantly higher binding activity to PkDBPαII compared to Fy(a+b-). Further studies need to be carried out to investigate the infection susceptibility of individuals with Fy(a+b+) and Fy(a+b-) erythrocytes by *P. knowlesi.*

Keywords: *Plasmodium knowlesi*, Duffy binding protein, Duffy antigen

IN VITRO EFFICACY OF ESSENTIAL OILS FROM PLANTS IN INHIBITING FUNGI CAUSING NAIL INFECTIONS

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Onychomycosis is a common fungal disease of the nails which can cause impairment or loss of tactile function. Essential oils may be an alternative, novel anti-mycotic agent to treat onychomycosis due to the developing fungal resistance to current antifungal drugs. The aim of this study was to determine the efficacy of essential oils in inhibiting fungi causing nail infection. In the disc diffusion assay, 100 µl of each fungal suspension of *Trichophyton mentagrophytes, Fusarium solani, Aspergillus niger* and *Candida albicans* ($1 – 5 \times 10^6$ CFU mL^{-1}) was spread onto individual PDA plates and 6 mm Whatman discs impregnated with 10 µl of differing concentrations of essential oil consisting of 5%, 10%, 25%, 50%, 75%, 90%, 95% and 100% of eucalyptus and lemongrass oil were mounted onto the lawn of fungus on agar surface. From the assay, eucalyptus oil showed fungal inhibition at concentrations of 10 % and 5% whereas lemongrass oil showed inhibition at higher concentration at 75% and 5% when tested against *T. mentagrophytes* and *F. solani* respectively. For *A. niger* and *C. albicans,* eucalyptus oil showed inhibition at concentration of 95% and 50% respectively but there was no inhibition with the lemongrass oil. In the MIC test, eucalyptus oil showed the minimum inhibitory concentration at 5% for both *T. rubrum* and *F. solani* and at 10% for both *A. niger* and *C. albicans* while lemongrass oil showed the minimum inhibitory concentration at 75%, 5% and 50% for *T. rubrum*, *F. solani,* and *A. niger* respectively, and no inhibition for *C. albicans*. The potential for synergism with both the essential oils was tested against all four fungal species, however indifference was noted for the combined activity of both oils. Therefore, the use of eucalyptus oil alone at a concentration of 20% may be a potential therapy for onychomycosis due to its higher *in vitro* fungal inhibitory activity.

Keywords: Onychomycosis, Eucalyptus oil, Lemongrass oil

COMPARATIVE STUDY OF THE EFFICACY OF MANUAL EGGS COUNTING WITH MOBILE AUTOMATED COUNTING APPLICATION ON *AEDES* MOSQUITO POPULATION IN 17TH COLLEGE OF UNIVERSITI PUTRA MALAYSIA

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Dengue fever is endemic to Malaysian and in at least 100 countries in Asia, the Pacific, the America, Africa, and the Caribbean. Statistics in Malaysia shows that 39,379 number of dengue cases with 63 number of dengue-related deaths have occurred until April 2019. *Aedes aegypti* and *Aedes albopictus* are adaptive vectors that have a long lifespan. AedesTech Mosquito Home System (AMHS) is a vector control method which uses the "lure and kill" concept to control *Aedes* mosquito population. The efficacy of AMHS was tested at the 17th College of Universiti Putra Malaysia (UPM) in comparison with other commercial ovitrap. The outcome proved that AMHS effectively lowered the hatching eggs laid by the adult mosquito and thus, lowering the population. To obtain statistics, it is important to count the number of *Aedes* mosquito eggs present in the ovitrap. Therefore, automated technology have been introduced. This study aims to compare the efficacy of mosquito eggs manual counting with mobile automated count application on *Aedes* mosquito population in 17th College, UPM using AMHS. AMHS was placed along the stairs at each level of Block A, Block B, Block C and Block D of 17th College, UPM. The eggs laid by *Aedes* mosquito on the tissue inside the AMHS was counted manually and via mobile application to evaluate the accuracy of the application by capturing the image of the tissue parallel to the plane of the eggs. The number of *Aedes* mosquito eggs were digitalized and counted by the mobile application. Next, the tissue was placed under a magnifying glass and the number of eggs were counted manually. The mean value of auto-detection (127.54 ± 374.79) was significantly higher than the mean value of manual counting (22.48 ± 38.34). The mobile application has a low accuracy.

Keywords*: Aedes* mosquito, AMHS, automated count

EFFECTIVENESS OF COMMERCIALLY AVAILABLE CONTACT LENS DISINFECTING SOLUTIONS AGAINST CLINICAL AND ENVIRONMENTAL ISOLATES OF *ACANTHAMOEBA* SPP.

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Acanthamoeba is an opportunistic protozoa free living that is well distributed in environment including air, soil, freshwater, seawater, tap water, bottle mineral, laboratory distilled water, chlorinated swimming polls and sewage. Contact lens wear is a main risk factor for keratitis caused by *Acanthamoeba* and the number of cases are increasing. This study was conducted to investigate and compare the effectiveness commercially available of contact lens disinfecting solution against clinical and environment isolates of *Acanthamoeba*. Two brands of commercially available contact lens disinfecting solution were used to examine their efficacy act as anti-*Acanthamoeba*; Oxysept and Opti Free. Cyst suspension of each strain was prepared and grown on Non-nutrient agar (NNA) for 11 days at 30°C. The cyst suspension of each strain was tested against each contact lens solution based on manufacturer recommendations for 4 hours, 6 hours and 8 hours. After the soaking time, 100µl cyst suspension was taken and cultured on NNA seeded with heat-killed *E. coli* at 30°C and observed daily under an inverted microscope for 14 days to detect the presence of trophozoite. The presence of trophozoite indicates contact lens disinfecting solutions are not effective act as anti-*Acanthamoeba*. Oxysept step showed a very good effect as anti-*Acanthamoeba* on all the *Acanthamoeba* strains tested within the testing time. Opti Free was ineffective at inactivating the *Acanthamoeba* cyst within the testing time despite claims of having anti-*Acanthamoeba* property. Contact lens disinfecting solution with hydrogen peroxide is effective as anti-*Acanthamoeba* agent and recommended for the prevention of *Acanthamoeba* keratitis.

Keywords: Risk Factor, Oxysept and Opti Free, *Acanthamoeba* Keratitis

ANTIBACTERIAL EFFECT OF *SYNSEPALUM DULCIFICUM* LEAVES EXTRACT AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA.

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Synsepalum dulcificum is an interesting plants that are known with its miracle berries which can alter the sourness into sweetness. The leaves also caused mutation and oxidative damaged. The antibacterial effect of crude methanol extracts of miracle leaves, *S. dulcificum* against Gram positive and Gram negative bacteria was studied as part of the effort in developing new antibacterial agent. Extraction of the sample using methanol was employed to obtain the crude extract. Susceptibility test against 6 strains of bacteria was carried out using the agar well diffusion method. Crude extract with the concentration of 100 mg/ml, 50 mg/ml and 20 mg/ml were pipetted in 9 mm diameter agar well with 50 µl in volume/well. Methanol crude extract at 100 mg/ml and 50 mg/ml concentration showed zone of inhibition as a result towards *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus*. However, no zone of inhibition was observed against Gram negative bacteria , i.e., *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella spp*. Another test was also conducted using Minimum Inhibitory Concentration test (MIC). The lowest minimum inhibitory concentration (MIC) value was exhibited by the crude extract against *S. aureus*, *S. epidermidis* and *B. cereus*, 50, 50 and 100 µg/well respectively. The morphological changes of the bacteria after being treated with the leaves extract were also observed under scanning electron microscope (SEM). The result suggested *S. dulcficum* leaves extract exhibit antibacterial effect on the highest concentration.

Keywords: Antibacterial, *Synsepalum dulcificum*, SEM

COMPARISON OF MICROORGANISM LOADS ISOLATED FROM THE LIFT BUTTONS OF SIX FACULTIES IN IIUM KUANTAN

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IIUM Kuantan Campus houses six faculties designated as "kulliyyahs" namely Kulliyyah of Allied Health Sciences (KAHS), Kulliyyah of Dentistry (KOD), Kulliyyah of Medicine (KOM), Kulliyyah of Nursing (KON), Kulliyyah of Pharmacy (KOP) and Kulliyyah of Science (KOS). Students from all faculties are involved with microbiological specimens and laboratory practical. Improper hygienic practice might cause microorganism transmission especially to shared facilities such as lift buttons. This study aims to compare the microorganism loads isolated from the lift buttons of six faculties in IIUM Kuantan and to characterise the microorganism via morphology observation and Gram type. Swab samples were collected aseptically from 295 lift buttons of six faculties followed by quantification via plate count technique. Viable colonies were cultured on nutrient, mannitol salt and MacConkey agar for morphology observation. Gram staining and microscopy examination were performed for further characterisation of microorganism. KAHS represents the highest occurrence of microorganism growth in which 100% of the samples isolated showed positive microorganism colonisation. Other faculties also demonstrated a high occurrence of microorganism growth with more than 80% of the samples had microorganism grown on plate. The highest microorganism loads was derived from KOM followed by KOD, KON, KAHS, KOS and KOP with 19400, 8640, 5160, 2780, 432 and 376 CFU/ml, respectively. KOM showed the highest microorganism loads for both interior and exterior buttons with 11700 and 7700 CFU/ml, respectively. Morphology observation and Gram type classification successfully grouped all isolates into 29 clusters. All clusters were Gram positive except one cluster was a fungus. PCR amplification of the 16S rDNA and 18 rDNA is currently underway to identify the isolates to the species level. In conclusion, KOM harboured the highest microorganisms on the lift buttons as compared to other faculties. This preliminary finding may support the hypothesis of lift buttons that served as fomite.

Keywords: Lift, fomites, quantification, CFU, morphology

SEQUENCE CONSERVATION ANALYSIS OF LIPL21 MRNA, AMINO ACID AND B-CELL EPITOPE IN DIFFERENT *LEPTOSPIRA* STRAINS USING BIOINFORMATICS APPROACH

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Issues in diagnosis of leptospirosis based on clinical manifestation continuous to be a critical concern for physicians since early 20th century due to its ability to imitate other pyretic diseases. Therefore, laboratory tests are essential to confirm the diagnosis of clinically suspected leptospirosis. However, lack of sensitive molecular markers for acute leptospirosis have led to inaccurate diagnostic techniques. Accessibility of complete genome sequence databases for different *Leptospira* strains drives the discovery of putative virulence factors for the development of reliable diagnostic tools for leptospirosis. LipL21 has been identified as a candidate diagnostic marker for leptospirosis, yet it lacks specificity due to the newly emerging pathogenic serovars. Thus, LipL21 in all serovars need to be studied in depth to understand the pathogenesis of leptospirosis to facilitate early detection. This study was aimed to evaluate the suitability of LipL21 as a leptospiral molecular marker by identifying the degree of sequence conservation in predicted mRNA nucleotides, amino acid and B-cell epitope of different *Leptospira* serovars and strains. Location of LipL21 conserved regions and B-cell epitope in 21 pathogenic and two non-pathogenic strains of five *Leptospira* species, were identified using bioinformatics database and tools such as National Center of Biotechnology, Rapid Annotation Subsystem Technology, Muscle program, Jalview software, SignalP 4.0, LipoP 4.1, ABCPred and BepiPred 2.0. Multiple sequence alignment analysis revealed that two LipL21 mRNA conserved regions were observed in 10 pathogenic *Leptospira* strains from nucleotide position 29 to 53 and 100 to 137, however conserved amino acid sequences corresponding to mRNA sequences were found in all pathogenic strains (111-149 and 155-192). The distinction between gene and amino acid results is due to the degenerate genetic code feature. Pairwise alignment revealed that the predicted LipL21 amino acid sequences among pathogenic *Leptospira* strains exhibited more than 93% sequence identity. Seven conserved B-cell epitopes were predicted from LipL21. Our findings suggest that LipL21 protein and its B-cell epitope have the potential to be used as diagnostic markers for detection of *Leptospira* pathogens compared to LipL21 mRNA.

Keywords: leptospirosis, *Leptospira*, *lipL21*, serovar, bioinformatics, B-cell epitope

THE INTERACTION BETWEEN METHANOL LEAVES EXTRACTS OF *Synsepalum dulcificum* WITH PENICILLIN G POTASSIUM AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Recently, infectious disease caused by the bacteria becomes difficult to control due to bacterial resistant factor. Penicillin that was introduced by Alexander Fleming was becoming increasingly insensitive towards controlling bacterial infection. Therefore, it is important to develop a new approach for antimicrobial treatment against drug-resistant bacteria. The objective of this study is to scrutinize a new strategy using synergistic activity between the natural product with an antibiotic action in order to control the bacterial infection. The study involved the combination between methanol leaves extracts of *Synsepalum dulcificum* with penicillin G potassium against Gram-positive and Gram-negative bacteria. The bacterial strains that have been used were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The crude extract of *S. dulcificum* was extracted using methanol cold extraction in ratio 1:10. The sensitivity test between methanol leaves extracts of *S. dulcificum* combined with penicillin G potassium is observed by applying the standard agar well diffusion method. Different types of concentration have been used to observe the action of the combination between methanol leaves extracts of *S. dulcificum* and penicillin G potassium. Besides from antimicrobial effect, scanning electron microscopy (SEM) is used to observe the ultra-structure changes that occur on the bacteria in response to the action of the combination between methanol leaves extracts of *S. dulcificum* with penicillin G potassium. As a result, the well diffusion method showing there is antagonist interaction of combination between methanol leaves extracts of *S. dulcificum* with penicillin G potassium against all the bacteria that been tested in this study. The result shows that the methanol extract of *S. dulcificum* have weak antimicrobial activity against the Gram-negative bacteria. As a conclusion, the methanol leaves extracts of *S. dulcificum* with penicillin G potassium shows good inhibition against Gram-positive but no antibacterial activity against Gram-negative bacteria.

Keywords: Methanol leaves extracts of *S. dulcificum*; agar well diffusion method; scanning electron microscopy (SEM)

MOLECULAR CHARACTERIZATION OF *STREPTOCOCCUS PNEUMONIAE* ISOLATES FROM CLINICAL ORIGIN

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Streptococcus pneumoniae (pneumococcus) colonizes human nasopharynx and causes severe infection including otitis media, pneumonia, bacteremia, and meningitis. However, there is still limited data on the genotypic characteristics of pneumococcal isolates from Malaysian settings. Therefore, this study was conducted to characterize clinical pneumococcal isolates from two Malaysian tertiary hospitals for presence of virulence genes (including pili), serotype, antibiotic susceptibility pattern and genomic background. A total of 60 pneumococcus isolates were collected from a hospital in Klang valley and another hospital in east coast. The appearance of alpha-hemolysis and greenish background on Blood agar, optochin susceptibility test and bile solubility test were used to identify *S. pneumoniae*. Disk diffusion method was applied to determine antibiotic susceptibility and further assessed by E-test for resistant strains. Polymerase Chain Reaction (PCR) was performed for screening virulence genes (*ply, lytA, pspA, cbpA, pavA* and for pili; *rlrA, RrgA, RrgC, SipA*) while Multiplex PCR was performed to determine serotype of isolates. Genetic fingerprint of pneumococcal isolates was generated by BOX PCR. Most of the isolates were resistant to penicillin (58.3%), tetracycline (46.7%) and erythromycin (40%). Eight out of sixty isolates exhibited multidrug-resistance (MDR). All isolates were detected for the virulence genes but only 36.7% of isolates had pili genes. The serotype distribution is 6A/B (23.3%), 19A (20%), 19F (15%) and 14 (8.3%). Based on BOX PCR analysis, six clusters and three single isolates were generated at a genetic distance percentage of 20.5% distinguishing most of the isolates according to origin of hospitals. These findings accumulate information on the phenotypic and genotypic properties of pneumococcal isolates which is useful for epidemiological comparison with other studies.

Keywords: *Streptococcus pneumoniae,* serotype, BOX PCR

ENUMERATION AND CHARACTERISATION OF MICROORGANISMS ISOLATED FROM THE LIFT BUTTONS OF A HOSTEL IN IIUM KUANTAN

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Mahallah Khalid Al-Walid (MKAW), a ten-storey hostel which consisted of four blocks (A, B, C and D) is the only hostel being occupied by male residents from all faculties in IIUM Kuantan Campus. Students from all faculties with medical, health sciences and science background dealt with microbiological samples and pathogenic materials during their lab and clinical sessions. It is predicted that students might transmit microorganism from their faculties to the hostel. Lift buttons are hypothesised to be the potential fomites since lift was shared by all residents from various hygienic conditions. Up to now, study of lift buttons as fomites especially in science-based academic setting and students' hostel in Malaysia is still lacking. Hence, this study was conducted to enumerate the microorganism isolated from the lift buttons of MKAW followed by characterisation of the microorganism by morphological observation on selected media and Gram type. A total of 88 lift buttons were randomly swabbed from eight lifts and cultured on nutrient, mannitol salt and MacConkey agar for enumeration and isolation. The isolates were then characterised based on morphological characteristics on media and Gram type classification. Out of 88 lift button samples collected, 95.5% indicated the presence of microorganism whereby lift buttons in Block A showed the highest quantity of microorganism followed by Block C, B and D with 49 970, 25 680, 4350 and 2790 CFU/ml, respectively. According to morphology characteristics and Gram type, 56 groups of microorganisms have been classified in which 2 groups belong to fungi. Out of 54 bacterial groups, Gram-positive cocci and Gram-positive diplococci dominated the isolates followed by Gram-positive diplobacilli, Gram-positive bacilli, Gram-positive coccobacilli and Gram-negative bacilli. Further identification of microorganism up to the species level will be done via molecular approach. Conclusively, the results obtained supported the hypothesis that lift buttons are source of fomites.

Keywords: Lift buttons, fomites, Gram staining

SUSCEPTIBILITY OF *ACANTHAMOEBA SPP.* CYSTS FROM CLINICAL AND ENVIRONMENTAL STRAINS TOWARDS ANTIMICROBIAL AGENTS

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Acanthamoeba Keratitis cases are in the increase globally especially among the contact lens users. This ocular disease happens due to the infection of *Acanthamoeba sp*. parasites. The difficulties in providing exact effective treatment to the patients occurs as the cysts of *Acanthamoeba sp*. are very resistant towards antimicrobial agents. Study is done to observe the sensitivity of the isolated clinical and environmental strains of *Acanthamoeba sp* (HKL 55, HS 19, OT7(1) and OF7) towards the antimicrobial agents at therapeutic doses whereby *chlorhexidine* 0.02% and *propamidinine isethionate* (Brolene) 0.1%. The Minimum Cysticidal Concentration (MCC) for the antimicrobial has also been identified. Two fold serial dilution has been performed on microtiter plate using page amebic saline solution for both the antimicrobial agents in the concentrations of 200 μg/ml to 0.0977 μg/ml for chlorhexidine and 1000 μg/ml to 0.4883 μg/ml for *propamidine isethionate.* The *Acanthamoeba* cysts are exposed to the antimicrobial solutions for 24hours and the cysts on the vial are rinsed and centrifuged to remove any remaining antimicrobial residues. The sediment that contains cysts are cultured onto non-nutrient agar layered with heat-killed *E.coli*. The cultures are observed under inverted microscopes for fourteen days to see any excystment of the cysts. Both the antimicrobial agents has showed cysticidal effects at therapeutic doses. The range of MCC for *chlorhexidine* is from 25 μg/ml to 50 μg/ml and 62.5 μg/ml to 500 μg/ml for *propamidine isethionate.* The MCC mean value for *chlorhexidine* and *propamidine* are 31.25 ± 12.5 μg/ml and 187.5 ± 210.41 μg/ml respectively. The difference in the cysticidal effects of *chlorhexidine* and *propamidine isethionate* is statistically significant where ($p < 0.05$). The *invitro* sensitivity test carried out could determine the MCC values and also identify any resistance of the *Acanthamoeba* cysts towards antimicrobial agents.

Keywords: Susceptibility, *Acanthamoeba spp*, antimicrobial agent

ANTIFUNGAL EFFECTS OF LEMONGRASS AND EUCALYPTUS ESSENTIAL OILS ON FUNGI CAUSING NAIL INFECTIONS

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Onychomycosis is a fungal nail infection where the common fungi that contribute to the infection are dermatophytes and non-dermatophytes fungi. Onychomycosis can be treated using antifungal drugs, yet resistance can develop against antifungal agents thus a recurrence of nail infection is high. Treatment using natural products such as essential oils may have higher efficacy in treating onychomycosis. The aim of this study was to assess in vitro and ex vivo antifungal activity of essential oils as a potential alternative treatment for fungal nail infections. Commercially prepared lemongrass and eucalyptus essential oils were tested against two species of fungi, dermatophyte *Trichophyton mentagrophytes* and yeast *Candida albicans*. Disc diffusion and microdilution assays were conducted to determine the inhibition zones and minimum inhibitory concentration (MIC) of the essential oils against the fungi. The essential oils were also used to treat ex vivo infected nail samples to determine their efficacy in reducing the fungal load in nails. The result for disk diffusion assay showed inhibition of both fungus at 10% concentration of eucalyptus essential oil while lemongrass essential oil at 100% concentration only inhibited *Trichophyton mentagrophytes*. In MIC tests, eucalyptus essential oil inhibited both *Trichophyton mentagrophytes* and *Candida albicans* at 6.25% and 12.5% concentration respectively but lemongrass essential oil inhibited *Trichophyton mentagrophytes* only at the concentration of 100%. The ex vivo interpretation of eucalyptus and lemongrass essential oils showed 100% reduction of *Trichophyton mentagrophytes* fungal load in nails after 14 days of treatment while eucalyptus essential oil showed 92.9% reduction of *Candida albicans* fungal load in infected nails after the second week of treatment. In conclusion, eucalyptus essential oil can be used as an alternative treatment for onychomycosis due to its capability of antifungal activity even at low concentrations.

Keywords: antifungal agents; essential oils; onychomycosis