ISOLATION, DETECTION AND GENOMIC DIFFERENTIATION OF *Escherichia coli* FROM AQUATIC ENVIRONMENTS IN KELANTAN, MALAYSIA.

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ABSTRACT

Diarrhea caused by *Escherichia coli* is one of the main diseases associated with water supply and sanitation. The main aims of this study were to isolate and confirm the presence of *E. coli* in selected aquatic environments in Bachok, Kelantan, as well as to determine the incidence of their virulence genes and the genomic diversity among the isolates. Fifty water samples from various aquatic environments of Bachok, Kelantan were examined to determine their microbiological quality by applying both phenotypic and genotypic methods for detection of total coliform and *E. coli*. The presence of total coliform was significantly correlated to *E. coli* ($p<0.05$). Based on biochemical tests, 78% of the samples had *E. coli* with an average density of 1×10^6 cfu/100mL. Among the 39 isolates recovered, 74% (29 isolates) of *E. coli* were positive for the *phoA* gene, which is the housekeeping gene for *E. coli*. A hexaplex PCR was performed to detect six virulence genes in pathogenic *E. coli* using 5 sets of primers (ST1, LT1, LT2, VT and AE). *E. coli* from only one sample (EC15) was positive for the LT1 gene, which codes for LT-heat labile toxin. Antimicrobial susceptibility tests (AST) showed that only ETEC isolate was resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. The rest of *E. coli* isolates were susceptible to the tested antibiotics. The analysis of genomic diversity of *E. coli* isolates by Repetitive Extragenic Palindromic (REP)-PCR generated 27 patterns (F=0.26-1.0). The REP-PCR profiles were reproducible and the multiple DNA fingerprints showed that the *E. coli* isolates were genetically diverse. A dendrogram generated by the UPGMA algorithm showed 4 clusters of *E. coli* isolates based on 80% similarity. Overall, REP-PCR generated high genetic variability within the *E. coli* isolates. The finding in the study indicates that

REP-PCR is a promising molecular method for determining the genomic diversity of environmental *E. coli* strains.

ABSTRAK

Penyakit cirit-birit disebabkan oleh *Escherichia coli* adalah salah satu penyakit utama yang dikaitkan dengan bekalan air dan sanitasi. Tujuan utama kajian ini adalah untuk mengasingkan dan mengesahkan kehadiran *E. coli* dalam persekitaran air terpilih di Bachok, Kelantan, serta untuk mengesan kehadiran gen-gen virulen and kepelbagaian genom diantara isolate *E. coli*. Lima puluh sampel air dari pelbagai persekitaran Bachok, Kelantan telah diperiksa bagi menentukan kualiti mikrobiologi dengan menggunakan kaedah fenotip dan genotip untuk pengesanan jumlah total coliform dan *E. coli*. Kehadiran jumlah total coliform adalah dihubung kait nyata sekali kepada *E. coli* ($p<0.05$). Berdasarkan ujian biokimia, 78% daripada sampel yang diperolehi mengandungi *E. coli* dengan purata ketumpatan 1 x 10^6 cfu/100mL. Antara 39 isolat yang dikesan, 74% (29 isolat) *E. coli* adalah positif untuk kehadiran gen *phoA* yang merupakan gen 'housekeeping' untuk *E. coli*. Satu ujian heksapleks PCR telah diusahakan bagi mengesan enam gen virulen dalam *E. coli* patogenik menggunakan 5 set primer (ST1 , LT1 , LT2 , VT dan AE). *E. coli* daripada hanya satu sampel (EC15) adalah positif untuk gen LT1, yang mengekod untuk toksin LT-heat labile. Ujian sensitiviti antibiotik (AST) menyatakan yang hanya ETEC menunjukkan ketahanan terhadap ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. Isolat *E. coli* yang lain tidak menunjukkan sebarang ketahanan terhadap antibiotik yang diuji. Analisis diversiti genom isolat *E. coli* oleh Repetitive Extragenic Palindromic (REP)-PCR menghasilkan 27 profil (F=0.26 1.0). Profil REP PCR mampu dihasilkan semula dan fingerprint DNA berbilang menunjukkan bahawa isolat *E. coli* mempunyai diversiti yang tinggi. Dendrogram yang dihasilkan oleh algoritma UPGMA menunjukkan 4

cluster isolat *E. coli* berdasarkan 80% persamaan. Keseluruhannya, REP PCR menghasilkan variasi genetik yang tinggi dalam isolat *E. coli*. Penemuan dalam kajian itu menunjukkan yang REP-PCR adalah satu kaedah molekular yang baik untuk penentuan diversiti genom *E. coli* dari alam sekitar.

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- Poster presented at the South China Sea Conference (SCS), Kuantan Pahang on 25th -29th Nov 2008.

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