

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 1×10^6 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 isolate *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 isolate *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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CONTENTS

Abstract	iii
Abstrak	v
Acknowledgments	vii
Publication and Presentation	viii
Contents	ix
List of Figures	xiv
List of Tables	xvi
Chapter 1: Introduction	
1.1 Background of the study	1
1.2 Objectives of the study	6
1.3 Scope of the study	6
Chapter 2: Literature review	
2.1 Water Quality and Sanitation	7
2.2 Microbial Contamination	7
2.3 Water quality indicator	8
2.4 <i>Escherichia coli</i>	9
2.5 Pathogenic <i>E. coli</i>	10
2.6 Molecular Approach	12
2.7 Detection of pathogenic strains	13
2.7.1 Phenotypic Assays	

2.7.2	Molecular Assays	
2.8	Microbial source tracking	15
2.8.1	Phenotypic source tracking	
2.8.1.1	Antibiotic resistance analysis (ARA)	
2.8.2	DNA based source tracking	
2.9	Rep-PCR	18
2.9.1	Advantages of rep-PCR Analysis	

Chapter 3: Materials and methods

3.1	Materials	21
3.1.1	Sampling	21
3.1.2	<i>E. coli</i> isolates	21
3.1.3	Media and reagents	22
3.1.3.1	Media for bacterial growth	
3.1.3.2	Materials for Biochemical Tests	
3.1.3.3	Other Solutions	
3.1.4	PCR Materials and Equipments	26
3.1.4.1	Materials and Equipments for PCR assays	
3.1.4.2	Materials and Equipments for Agarose Gel Electrophoresis	
3.2	Methods	31
3.2.1	Collection of water samples	31
3.2.2	Media preparation	31
3.2.3	Filtration of water samples	31

3.2.4 Isolation of <i>E. coli</i> and other coliforms	32
3.2.5 Biochemical tests	32
3.2.5.1 Indole test	
3.2.5.2 Methyl red test	
3.2.5.3 Voges Proskauer test	
3.2.5.4 Citrate utilization test	
3.2.5.5 Triple Sugar Iron (TSI) test	
3.2.6 API 20E	35
3.2.7 Total DNA extraction from bacterial culture	35
3.2.8 Polymerase Chain Reaction (PCR) assay	36
3.2.8.1 PCR material and master mixture preparations	
3.2.8.2 Confirmation of <i>E. coli</i> using Monoplex PCR assay	
3.2.8.3 Toxin genes detection using Multiplex PCR assay	
3.2.8.4 PCR conditions	
3.2.8.5 Running Agarose Gel Electrophoresis	
3.2.9 Antimicrobial agent susceptibility testing (ARA)	42
3.2.9.1 Preparation of inoculum	
3.2.9.2 Inoculation of Mueller-Hinton agar	
3.2.10 Repetitive extragenic palindromes (REP)-PCR amplification	
3.2.11 Interpretation of fingerprints	45

Chapter 4: Results

4.1 Sampling and isolation of total coliform and <i>E. coli</i>	46
4.2 Correlation between total coliform and <i>E. coli</i>	50

4.2.1 Comparison of total coliform and <i>E. coli</i> colony counts isolated at different holding time	52
4.3 Identification of <i>E. coli</i> isolates	54
4.3.1 Confirmation by using biochemical tests	54
4.3.2 Confirmation by using API 20E	56
4.3.3 Optimization of monoplex PCR using different primer concentration	57
4.3.3.1 Confirmation by using monoplex PCR assay	58
4.4 Virulence gene detection	61
4.4.1 Detection of <i>E. coli</i> 0157:H7 by selective plating	62
4.5 Antimicrobial susceptibility test of <i>E. coli</i> strains	63
4.6 Genetic diversity of <i>E. coli</i> using PCR based subtyping	64
4.6.1 Optimization of REP-PCR using different primers	64
4.6.2 REP-PCR of isolates	65
4.6.3 Cluster analysis of <i>E. coli</i> isolates	66
4.6.3 Comparison of REP-PCR of <i>E. coli</i> isolates from clinical and food samples	69
4.6.4 Cluster analysis of <i>E. coli</i> from different sources	73
Chapter 5: Discussion	
5.1 Isolation and identification of <i>E. coli</i>	74
5.2 Survival of <i>E. coli</i> and total coliform at different holding time	76
5.3 PCR confirmation of <i>E. coli</i> isolates	78
5.4 Prevalence of virulence genes	79
5.5 Antimicrobial Susceptibility test	80

5.6 Genomic diversity among isolates based on REP-PCR	81
Chapter 6: Conclusion	
6.1 Conclusion	83
References	84
Appendixes	102

LIST OF FIGURES

Fig. 1.1a	Sampling sites around Bachok, Kelantan.	3
Fig.4.1a	<i>E. coli</i> produce dark blue to violet colonies on CCA plates (arrow). Total coliform produce salmon to red colonies.	47
Fig. 4.1b	<i>E. coli</i> produce greenish metallic sheen colonies on EMB agar plates.	47
Fig. 4.2a	Correlation of total coliform and <i>E. coli</i> ($p < 0.05$)	51
Fig. 4.2b	Comparison of colony count of total coliform and <i>E. coli</i> isolated at different holding time.	53
Fig. 4.3a	<i>E. coli</i> negative for citrate utilization.	55
Fig. 4.3b	<i>E. coli</i> in TSI tubes produce an acid butt, an acid slant and gas.	55
Fig. 4.3c	<i>E. coli</i> was positive for indole production.	55
Fig. 4.3d	<i>E. coli</i> was positive for methyl red test.	55
Fig. 4.3e	API 20E kits shows the identification of <i>E. coli</i>	57
Fig. 4.3f	Representative gel of monoplex PCR for <i>phoA</i> gene detection of 11 presumptive <i>E. coli</i> isolates.	57
Fig. 4.3g	Representative gel of monoplex PCR for <i>phoA</i> gene detection of 10 presumptive <i>E. coli</i> isolates (primer- 0.1 μ M).	58
Fig. 4.4a	Representative gel showing multiplex PCR using 5 sets of primers.	62
Fig. 4.5a	Antimicrobial susceptibility test plates of sample 15 which was resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole.	63

Fig. 4.6a	Representative gel showing REP-PCR for three isolates (EC6, EC7, EC44) using four different types of primers.	64
Fig. 4.6b	Representative DNA fingerprints of 11 <i>E. coli</i> isolates generated by REP1R(b) primer.	66
Fig. 4.6c	Dendrogram showing the result of cluster analysis of the REP-PCR patterns from 29 isolates of <i>E. coli</i> generated with GelCompar software by the UPGMA method. The different fingerprint patterns and location of samples are indicated.	68
Fig. 4.6d	Representative DNA fingerprint of clinical and food <i>E. coli</i> isolates generated by REP 1R(b) primer.	70
Fig. 4.6e	Dendrogram showing the result of cluster analysis of the REP-PCR patterns from 49 isolates of <i>E. coli</i> generated with GelCompar software by the UPGMA method. The different fingerprint patterns and location of samples are indicated.	72

LIST OF TABLES

Table 3.1a	The bacterial control strains and their respective genes.	22
Table 3.1b	Primers used for detection of housekeeping gene (monoplex) and virulence genes (multiplex) of <i>E. coli</i> .	28
Table 3.1c	Primers used for REP-PCR assays.	29
Table 3.2a	Monoplex PCR master mixture.	38
Table 3.2b	Multiplex PCR master mixture.	38
Table 3.2c	PCR amplification condition for monoplex and multiplex PCR assays.	39
Table 3.2d	PCR amplification condition for REP- PCR assay.	44
Table 3.2e	REP-PCR Master mixture.	44
Table 4.1c	Colony count of total coliform and <i>E. coli</i> for fifty samples at two different holding time.	48
Table 4.2a	Pearson correlation of <i>E. coli</i> and total coliform.	50
Table 4.3a	Results for API 20E test.	56
Table 4.3b	Summary of <i>E. coli</i> recovery from different confirmation stages.	59
Table 4.6a	Sources and origin of clinical and food sample used.	69
Table 4.1a	Global positioning system (GPS) of fifty samples collected around Bachok, Kelantan.	101
Table 4.5a	Antimicrobial susceptibility test result of 29 <i>E. coli</i> strains.	103
Table 4.1b	Salinity and other detailed particulars of 15 samples	104

CHAPTER 1: INTRODUCTION