Chapter 1

General Introduction ......................................................... 01 - 17

1.1 Introduction ............................................................................................................ 01
  1.1.1 Escherichia coli ................................................................................................. 01
    1.1.1.1 E. coli as an emerging pathogen ............................................................... 04
    1.1.1.2 Enterotoxigenic E. coli (ETEC) .................................................................. 04
    1.1.1.3 Shiga toxin-producing E. coli (STEC) .......................................................... 06
    1.1.1.4 Enteropathogenic E. coli (EAEC) ............................................................... 08
    1.1.1.5 Enteroinvasive E. coli (EIEC) ..................................................................... 10
    1.1.1.6 Enteropathogenic E. coli (EPEC) ............................................................... 10
    1.1.1.7 Diffusely adherent E. coli (DAEC) ............................................................. 12
    1.1.1.8 Extraintestinal pathogenic E. coli (ExPEC) .. 13
  1.1.2 Serotyping ......................................................................................................... 14
  1.1.3 Phylogenetic analysis ......................................................................................... 14
  1.1.4 Antibiotic resistance ......................................................................................... 15
  1.1.5 Antibiotic resistance genes ............................................................................... 15
  1.1.6 Genotyping ....................................................................................................... 16
  
1.2 Broad objectives ..................................................................................................... 17

Chapter 2

Isolation, identification and serotyping of
Escherichia coli isolates from estuarine, seafood and clinical sources ................................................................. 19 - 60

2.1 Introduction ............................................................................................................ 19
2.2 Review of Literature ............................................................................................. 20
  2.2.1 Serotyping ......................................................................................................... 20
  2.2.2 EPEC ................................................................................................................ 23
  2.2.3 EIEC .................................................................................................................. 24
  2.2.4 EAEC ................................................................................................................ 25
  2.2.5 ETEC ................................................................................................................ 25
  2.2.6 STEC ................................................................................................................ 26
  2.2.7 UPEC and/or ExPEC ....................................................................................... 28
2.3 Objectives .............................................................................................................. 29
2.4 Materials and Methods ........................................................................................ 29
  2.4.1 Isolation of E. coli from Cochin estuary ............................................................ 29
    2.4.1.1 Description of the study area ..................................................................... 29
    2.4.1.2 Collection of samples ............................................................................... 29
    2.4.1.3 Isolation of E. coli from water samples ................................................. 32
2.4.2 Isolation of E. coli from seafood samples ........................................ 32
2.4.3 Clinical isolates of E. coli ............................................................. 33
2.4.4 Biochemical Identification of E. coli ........................................... 33
  2.4.4.1 Indole test ........................................................................ 33
  2.4.4.2 Methyl Red (MR) test ....................................................... 34
  2.4.4.3 Voges Proskauer (VP) test ................................................ 34
  2.4.4.4 Citrate test ....................................................................... 34
2.4.5 Serotyping of E. coli .................................................................... 35
2.4.6 Molecular characterization of E. coli ...................................... 35
  2.4.6.1 Isolation of DNA from E. coli ........................................ 35
  2.4.6.2 Polymerase Chain Reaction (PCR) for detection of
         uid A gene in E. coli ......................................................... 35
2.5 Results .................................................................................................. 36
  2.5.1 Diversity of E. coli serotypes in Cochin estuary ....................... 36
     2.5.1.1 Overall diversity of E. coli serotypes in Cochin estuary ........ 36
     2.5.1.2 Distribution of different classes of E. coli serotypes
           in Cochin estuary .............................................................. 39
     2.5.1.3 Diversity of E. coli serotypes in station 1 (Chitoor) .......... 40
           2.5.1.3.1 Distribution of different classes of E. coli
                      serotypes in station 1 (Chitoor) ......................... 41
     2.5.1.4 Diversity of E. coli serotypes in station 2 (Bolgatty) .......... 42
           2.5.1.4.1 Distribution of different classes of E. coli
                      serotypes in station 2 (Bolgatty) ....................... 43
     2.5.1.5 Diversity of E. coli serotypes in station 3 (Off Marine
           Science Jetty) ................................................................. 44
           2.5.1.5.1 Distribution of different classes of E. coli
                      serotypes in station 3 (Off Marine Science Jetty) .... 45
     2.5.1.6 Diversity of E. coli serotypes in station 4 (Thevara) ......... 46
           2.5.1.6.1 Distribution of different classes of E. coli
                      serotypes in station 4 (Thevara) ......................... 46
     2.5.1.7 Diversity of E. coli serotypes in station 5 (Barmouth) ....... 47
           2.5.1.7.1 Distribution of different classes of E. coli
                      serotypes in station 5 (Barmouth) ....................... 47
  2.5.2 Diversity of E. coli serotypes in seafood sources from Cochin
           estuary ............................................................................. 48
     2.5.2.1 Distribution of different classes of E. coli serotypes
           in seafood sources ............................................................ 49
  2.5.3 Diversity of E. coli serotypes in clinical sources from Cochin City ... 49
     2.5.3.1 Distribution of different classes of E. coli serotypes
           in clinical sources ............................................................ 50
Chapter 3

Phylogenetic analysis of *Escherichia coli* isolates from estuarine, seafood and clinical sources ............... 61 - 97

3.1 Introduction ................................................................. 61
3.2 Review of Literature ......................................................... 62
   3.2.1 Phylogenetic analysis .......................................................... 62
   3.2.2 *E. coli* phylogenetic groups .................................................. 64
      3.2.2.1 Phylogenetic group B2 and D (Pathogenic groups) .......... 66
      3.2.2.2 Phylogenetic group A and B1 (Non-Pathogenic groups) ......... 69
   3.2.3 Phylogenetic group distribution among *E. coli* isolates from environmental sources ........................................... 69
   3.2.4 Phylogenetic group distribution among *E. coli* isolates from food sources ................................................................. 70
   3.2.5 Phylogenetic group distribution among *E. coli* isolates from clinical sources ................................................................. 71
   3.2.6 Distribution of virulence genes among various phylogenetic groups of *E. coli* ................................................................. 73
   3.2.7 Phylogenetic group distribution and antibiotic resistance ......... 76
3.3 Objectives ................................................................. 78
3.4 Materials and Methods .......................................................... 78
   3.4.1 Isolation of DNA from *E. coli* .................................................. 78
   3.4.2 Phylogenetic analysis ................................................................. 79
   3.4.3 Statistical analysis ................................................................. 80
3.5 Results ................................................................. 80
   3.5.1 Phylogenetic group distribution of *E. coli* isolates from Cochin estuary ................................................................. 80
      3.5.1.1 Phylogenetic group distribution of *E. coli* isolates from different stations set at Cochin estuary ............. 81
      3.5.1.2 Relative distribution of various phylogenetic groups of *E. coli* isolates among different stations set at Cochin estuary ................................................................. 83
4.5.1.1 Overall antibiotic resistance of *E. coli* isolates from Cochin estuary ........................................................ 132

4.5.1.1.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from Cochin estuary ........................................ 134

4.5.1.2 Antibiotic resistance of *E. coli* isolates from station 1 (Chitoor) ........................................................... 135

4.5.1.2.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from station 1 (Chitoor) ..................... 135

4.5.1.3 Antibiotic resistance of *E. coli* isolates from station 2 (Bolgatty) .......................................................... 136

4.5.1.3.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from station 2 (Bolgatty) ........................... 137

4.5.1.4 Antibiotic resistance of *E. coli* isolates from station 3 (Off Marine Science Jetty) ..................................................... 138

4.5.1.4.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from station 3 (Off Marine Science Jetty) ..................... 139

4.5.1.5 Antibiotic resistance of *E. coli* isolates from station 4 (Thevara) .......................................................... 140

4.5.1.5.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from station 4 (Thevara) ........................... 140

4.5.1.6 Antibiotic resistance of *E. coli* isolates from station 5 (Barmouth) .......................................................... 141

4.5.1.6.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from station 5 (Barmouth) ........................... 142

4.5.1.7 Incidence of antibiotic resistance: a comparison from all stations in Cochin estuary ........................................... 143

4.5.1.8 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates: a comparison from all stations in Cochin estuary ............ 144

4.5.2 Prevalence of antibiotic resistance among *E. coli* isolates from clinical sources ........................................................ 147

4.5.2.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from clinical sources ........................................ 149
4.5.3 Antibiotic resistance among *E. coli* isolates from seafood sources.............................................................................................................. 150
4.5.3.1 Overall incidence of antibiotic resistance among various phylogenetic groups of *E. coli* isolates from seafood sources .................................................................. 151
4.5.4 Relative prevalence of antibiotic resistance of *E. coli* isolates from different sources ...................................................................................................................... 152
4.5.4.1 Relative incidence of antibiotic resistance in various phylogenetic groups of *E. coli* isolates from different sources (estuary, seafood and clinical) .................. 152
4.5.5 MAR Index and resistance patterns of *E. coli* isolates from Cochin estuary...................................................................................................................... 155
4.5.5.1 MAR Index and resistance patterns of *E. coli* isolates from station 1 (Chitoor) .................................................................................................................. 155
4.5.5.2 MAR Index and resistance patterns of *E. coli* isolates from station 2 (Bolgatty) .................................................................................................................. 156
4.5.5.3 MAR Index and resistance patterns of *E. coli* isolates from station 3 (Off marine Science Jetty) ................................................................. 157
4.5.5.4 MAR Index and resistance patterns of *E. coli* isolates from station 4 (Thevara) ........................................................................................................... 159
4.5.5.5 MAR Index and resistance patterns of *E. coli* isolates from station 5 (Barmouth) ........................................................................................................... 159
4.5.6 MAR Index and resistance patterns of *E. coli* isolates from clinical sources ........................................................................................................................... 160
4.5.7 MAR Index and resistance patterns of *E. coli* isolates from seafood sources ...................................................................................................................... 163
4.6 Discussion .................................................................................................................. 164
4.6.1 Antibiotic resistance of *E. coli* isolates from Cochin estuary ................................................................................................................................. 164
4.6.2 Antibiotic resistance of *E. coli* isolates from seafood sources ................................................................................................................................. 168
4.6.3 Antibiotic resistance among *E. coli* isolates from clinical sources ................................................................................................................................. 171

Chapter 5

Prevalence of antibiotic resistance genes in *Escherichia coli* isolates from estuarine, seafood and clinical sources ................................................................. 179 - 257

5.1 Introduction ........................................................................................................... 179
5.2 Review of literature .......................................................................................... 181
5.2.1 Antibiotic resistance genes ........................................................................... 181
5.2.1.1 TEM ................................................................. 181
5.2.1.2 blaCTX-M .......................................................... 184
5.2.1.3 Tetracycline resistance genes (tetA and tetB) .......... 187
5.2.1.4 Sulphonamide resistance genes (sul1 and sul2) ...... 190
5.2.1.5 Trimethoprim resistance genes (dhfrIa and dhfrVII) .. 191
5.2.1.6 Quinolone resistance genes .................................. 193
5.2.1.7 Aminoglycoside resistance genes ......................... 194
5.2.1.8 Chloramphenicol resistance genes (catI) ................. 195
5.2.1.9 Class 1 integrons ................................................. 196

5.3 Objectives ............................................................................ 199
5.4 Materials and Methods .......................................................... 200
  5.4.1 Isolation of DNA from E. coli ........................................... 200
  5.4.2 Isolation of plasmid DNA from E. coli ......................... 200
  5.4.3 PCR detection of antibiotic resistant genes ..................... 200
    5.4.3.1 Detection of blaTEM gene ...................................... 201
    5.4.3.2 Detection of blaCTX-M gene .................................. 202
    5.4.3.3 Detection of tetA and tetB gene .............................. 202
    5.4.3.4 Detection of sul1 and sul2 gene .............................. 203
    5.4.3.5 Detection of dhfrIa and dhfrVII gene ....................... 203
    5.4.3.6 Detection of strA .................................................. 204
    5.4.3.7 Detection of aphA2 .............................................. 204
    5.4.3.8 Detection of catI .................................................. 205
    5.4.3.9 Detection of integrase1 .......................................... 205
    5.4.3.10 Detection of class 1 integron variable regions ............ 205
    5.4.3.11 Statistical analysis .............................................. 206

5.5 Results ................................................................................... 206
  5.5.1 Prevalence of antibiotic resistance genes among E. coli
      isolates from Cochin estuary .............................................. 206
    5.5.1.1 Overall prevalence of antibiotic resistance genes among E. coli
            isolates from Cochin estuary ................................. 206
    5.5.1.1.1 blaTEM ......................................................... 207
    5.5.1.1.2 blaCTX-M ..................................................... 208
    5.5.1.1.3 Sulphonamide resistance genes (sul1 and sul2) ... 209
    5.5.1.1.4 Trimethoprim-resistant genes (dhfrIa and dhfrVII) 209
    5.5.1.1.5 Tetracycline resistance genes (tetA and tetB) ........ 210
    5.5.1.1.6 Aminoglycoside resistance genes (strA and aphA2) 210
    5.5.1.1.7 Chloramphenicol-resistant gene (catI) ................. 212
    5.5.1.1.8 Class 1 integrons and intI gene ......................... 212
5.5.1.2 Prevalence of antibiotic resistance genes among various phylogenetic groups of *E. coli* isolates from Cochin estuary ................................................................. 213

5.5.1.3 Occurrence of antibiotic resistance genes among *E. coli* isolates from station 1 (Chitoor, n = 47) ........ 214
5.5.1.3.1 Incidence of antibiotic resistance genes among various phylogenetic groups of *E. coli* isolates from station 1 (Chitoor) .......... 215

5.5.1.4 Occurrence of antibiotic resistance genes among *E. coli* isolates from station 2 (Bolgatty, n = 75) ........ 216
5.5.1.4.1 Incidence of antibiotic resistance genes among various phylogenetic groups of *E. coli* isolates from station 2 (Bolgatty) .......... 217

5.5.1.5 Occurrence of antibiotic resistance genes among *E. coli* isolates from station 3 (Off Marine Science Jetty, n = 69) ................................................................. 218
5.5.1.5.1 Incidence of antibiotic resistance genes among various phylogenetic groups of *E. coli* isolates from station 3 (Off Marine Science Jetty) ................................................. 219

5.5.1.6 Occurrence of antibiotic resistance genes among *E. coli* isolates from station 4 (Thevara, n = 49) ........ 220
5.5.1.6.1 Incidence of antibiotic resistance genes among various phylogenetic groups of *E. coli* isolates from station 4 (Thevara) .......... 221

5.5.1.7 Occurrences of antibiotic resistance genes among *E. coli* isolates from station 5 (Barmouth, n = 60) .... 222
5.5.1.7.1 Incidence of antibiotic resistance genes among various phylogenetic groups of *E. coli* isolates from station 5 (Barmouth) .......... 223

5.5.1.8 Spatial variation in incidence of antibiotic resistance genes among *E. coli* isolates from different stations in Cochin estuary ................................................................. 224
5.5.1.8.1 Distribution of antibiotic resistance genes among *E. coli* phylogenetic group ‘A’ isolates from different stations at Cochin estuary ................................................................. 225
5.5.1.8.2 Distribution of antibiotic resistance genes among *E. coli* phylogenetic group ‘B1’ isolates from different stations at Cochin estuary ................................................................. 226
5.5.1.8.3 Distribution of antibiotic resistance genes among *E. coli* phylogenetic group ‘B2’ isolates from different stations at Cochin estuary ............................................................. 227

5.5.1.8.4 Distribution of antibiotic resistance genes among *E. coli* phylogenetic group ‘D’ isolates from different stations at Cochin estuary ......................................................... 228

5.5.2 Occurrence of antibiotic resistance genes among *E. coli* isolates from seafood sources ............................................................................. 229

5.5.2.1 Incidence of antibiotic resistance genes in various phylogenetic groups of *E. coli* isolates from seafood sources ................................................................. 231

5.5.3 Occurrence of antibiotic resistance genes among *E. coli* isolates from clinical sources ............................................................................. 232

5.5.3.1 Incidence of antibiotic resistance genes in various phylogenetic groups of *E. coli* isolates from clinical sources ................................................................. 234

5.5.4 Relative prevalence of antibiotic resistance genes among *E. coli* isolates from different sources: a comparison ........................................ 235

5.5.5 Relative prevalence of antibiotic resistance genes among different phylogenetic groups of *E. coli* isolates from estuary, seafood and clinical sources ......................................................... 235

5.6 Discussion .............................................................................................. 239

5.6.1 Prevalence of antibiotic resistance genes among *E. coli* isolates from Cochin estuary ................................................................. 239

5.6.1.1 *blaTEM* ..................................................................................... 240

5.6.1.2 *blaCTX-M* .................................................................................. 241

5.6.1.3 Tetracycline resistance genes (*tetA* and *tetB*) ......................................................... 241

5.6.1.4 Aminoglycoside resistance genes (*strA* and *aphA2*) ......................................................... 241

5.6.1.5 Chloramphenicol-resistant gene (*catI*) ......................................................... 242

5.6.1.6 Sulphonamide resistance genes (*sul1* and *sul2*) ......................................................... 242

5.6.1.7 Trimethoprim-resistant genes (*dfrA1a* and *dfrVII*) ......................................................... 243

5.6.1.8 *Class 1 integrons* and *intI* gene ......................................................... 243

5.6.2 Prevalence of antibiotic resistance genes among *E. coli* isolates from seafood sources ................................................................. 247

5.6.2.1 *blaTEM* ..................................................................................... 247

5.6.2.2 *blaCTX-M* .................................................................................. 248

5.6.2.3 Sulphonamide resistance genes (*sul1* and *sul2*) ......................................................... 248

5.6.2.4 Tetracycline resistance genes (*tetA* and *tetB*) ......................................................... 249

5.6.2.5 Aminoglycoside resistance genes (*strA* and *aphA2*) ......................................................... 250
5.6.2.6 Chloramphenicol-resistant gene (catI) ........................................... 250
5.6.2.7 Trimethoprim-resistant genes (dhfrIa and dhfrVII) ................. 251
5.6.2.8 Class 1 integrons and intI gene ................................................. 251

5.6.3 Prevalence of antibiotic resistance genes among E. coli isolates from clinical sources ........................................................................ 252
5.6.3.1 blaTEM ......................................................................................... 252
5.6.3.2 blaCTX-M .................................................................................. 253
5.6.3.3 Sulphonamide resistance genes (sulI and sul2) ....................... 254
5.6.3.4 Tetracycline resistance genes (tetA and tetB) .......................... 255
5.6.3.5 Aminoglycoside resistance genes (strA and aphA2) .................. 255
5.6.3.6 Chloramphenicol-resistant gene (catI) ................................... 255
5.6.3.7 Trimethoprim-resistant genes (dhfrIa and dhfrVII) ............. 255
5.6.3.8 Class 1 integrons and intI gene .................................................. 256

Chapter 6
Prevalence of extraintestinal virulence factor genes in
Escherichia coli isolates from estuarine, seafood and clinical sources ................................................ 259 - 310
6.1 Introduction ............................................................................................ 259
6.2 Review of literature ................................................................................ 260
6.2.1 Extraintestinal pathogenic E. coli (ExPEC) ..................................... 260
6.2.2 Phylogenetic background and virulence factor genes ................. 264
6.2.3 Antibiotic resistance and virulence ............................................. 265
6.2.4 Virulence versus colonization factors ......................................... 266
6.3 Objectives ............................................................................................... 266
6.4 Materials and Methods ........................................................................... 267
6.4.1 Isolation of DNA from E. coli .................................................. 267
6.4.2 Isolation of plasmid DNA from E. coli ...................................... 267
6.4.3 Phylogenetic analysis ................................................................. 267
6.4.4 Antibiotic susceptibility testing .................................................. 267
6.4.5 Detection of antibiotic resistant genes ....................................... 267
6.4.6 Detection of virulence factor genes .......................................... 267
6.4.7 Statistical analysis ......................................................................... 269
6.5 Results .................................................................................................... 269
6.5.1 ExPEC isolates from cochin estuary .......................................... 269
  6.5.1.1 ExPEC and distribution of virulence factor genes in cochin estuary .......................................................... 269
  6.5.1.1.1 ExPEC and phylogenetic groups ..................................... 269
  6.5.1.1.2 Virulence factor genes .................................................. 270
6.5.1.3 Relative prevalence of various virulence factor genes among different phylogenetic groups of *E. coli* isolates from Cochin estuary ............................................................ 271

6.5.1.4 Prevalence of antibiotic resistance among ExPEC isolates from Cochin estuary ............................................................. 273

6.5.1.5 Prevalence of antibiotic resistance genes among ExPEC isolates from Cochin estuary ............................................................. 276

6.5.1.2 ExPEC and distribution of virulence factor genes in station 1 (Chitoor) ....................................................... 278

6.5.1.2.1 ExPEC and phylogenetic groups ........................................ 278

6.5.1.2.2 Virulence factor genes ................................................. 279

6.5.1.2.3 Incidence of virulence factor genes among various phylogenetic groups of *E. coli* isolates from station 1 (Chitoor) ......... 279

6.5.1.3 ExPEC and distribution of virulence factor genes in station 2 (Bolgatty) ..................................................... 280

6.5.1.3.1 ExPEC and phylogenetic groups ..................................... 281

6.5.1.3.2 Virulence factor genes .................................................. 281

6.5.1.3.3 Incidence of virulence factor genes among various phylogenetic groups of *E. coli* isolates from station 2 (Bolgatty) ...... 281

6.5.1.4 ExPEC and distribution of virulence factor genes in station 3 (Off Marine Science Jetty) ...................................................... 283

6.5.1.4.1 ExPEC and phylogenetic groups ..................................... 283

6.5.1.4.2 Virulence factor genes .................................................. 283

6.5.1.4.3 Incidence of virulence factor genes among various phylogenetic groups of *E. coli* isolates from station 3 (Off Marine Science Jetty) ...................................................... 284

6.5.1.5 ExPEC and distribution of virulence factor genes in station 4 (Thevara) ...................................................... 285

6.5.1.5.1 ExPEC and phylogenetic groups ..................................... 285

6.5.1.5.2 Virulence factor genes .................................................. 286

6.5.1.5.3 Incidence of virulence factor genes among various phylogenetic groups of *E. coli* isolates from station 4 (Thevara) ...... 286

6.5.1.6 ExPEC and distribution of virulence factor genes in station 5 (Barmouth) ...................................................... 287

6.5.1.6.1 ExPEC and phylogenetic groups ..................................... 287
6.5.1.6.2 Virulence factor genes ........................................... 288
6.5.1.6.3 Incidence of virulence factor genes among various phylogenetic groups of *E. coli* isolates from station 5 (Barmouth) .................... 288
6.5.1.7 Relative prevalence of virulence factor genes in *E. coli* isolates from different stations in Cochin estuary ................................................................. 289

6.5.2 ExPEC and distribution of virulence factor genes in *E. coli* isolates from seafood sources ................................................................. 290
6.5.2.1 ExPEC and phylogenetic groups ..................................... 290
6.5.2.2 Virulence factor genes ................................................. 290
6.5.2.3 Incidence of virulence factor genes among various phylogenetic groups of *E. coli* isolates from seafood isolates ......................................... 291
6.5.2.4 ExPEC and antibiotic resistance ...................................... 292

6.5.3 ExPEC and distribution of virulence factor genes in *E. coli* isolates from clinical sources ................................................................. 292
6.5.3.1 ExPEC and phylogenetic groups ..................................... 292
6.5.3.2 Virulence factor genes .................................................. 293
6.5.3.3 Incidence of virulence factor genes among various phylogenetic groups of *E. coli* isolates from clinical sources ......................................... 293
6.5.3.4 Prevalence of antibiotic resistance among ExPEC isolates from clinical sources ................................................................. 295
6.5.3.5 Prevalence of antibiotic resistance genes among ExPEC isolates from clinical sources ................................................................. 297

6.5.4 Relative prevalence of virulence factor genes in *E. coli* isolates from different sources ................................................................. 298

6.6 Discussion ............................................................................. 299
6.6.1 ExPEC isolates from Cochin estuary .................................... 299
6.6.1.1 Virulence factor genes .................................................. 299
6.6.1.2 ExPEC and phylogenetic groups ..................................... 301
6.6.1.3 ExPEC and antibiotic resistance ...................................... 302

6.6.2 ExPEC isolates from seafood sources .................................. 304
6.6.2.1 Virulence factor genes .................................................. 304
6.6.2.2 ExPEC and phylogenetic groups ..................................... 305
6.6.2.3 ExPEC and antibiotic resistance ...................................... 306

6.6.3 ExPEC isolates from clinical sources .................................. 307
6.6.3.1 Virulence factor genes .................................................. 307
6.6.3.2 ExPEC and phylogenetic groups ..................................... 308
6.6.3.3 ExPEC and antibiotic resistance ...................................... 309
Chapter 7
Genotyping of *Escherichia coli* isolates from estuarine, seafood and clinical sources using ERIC-PCR, RAPD and RFLP ........................................... 311 - 345

7.1 Introduction ........................................................................................................ 311
7.2 Review of literature ............................................................................................ 312
  7.2.1 ERIC-PCR.................................................................................................... 312
  7.2.2 RAPD ......................................................................................................... 313
  7.2.3 RFLP .......................................................................................................... 314
7.3 Objectives ........................................................................................................... 314
7.4 Materials and Methods ...................................................................................... 315
  7.4.1 Isolation of DNA from *E. coli* ................................................................ 315
  7.4.2 PCR amplification of 16S rRNA gene ..................................................... 315
  7.4.3 Genotyping of *E. coli* isolates using ERIC-PCR .................................. 315
  7.4.4 Genotyping of *E. coli* isolates using RAPD .......................................... 316
  7.4.5 Genotyping of *E. coli* isolates using RFLP .......................................... 316
  7.4.6 Cladogram construction .......................................................................... 317
7.5 Results ................................................................................................................ 317
  7.5.1 Enterobacterial repetitive intergenic consensus (ERIC) PCR of *E. coli* isolates ......................................................................................... 317
    7.5.1.1 ERIC PCR of *E. coli* isolates from Cochin estuary ......................... 319
    7.5.1.2 ERIC PCR of *E. coli* isolates from seafood sources ....................... 319
    7.5.1.3 ERIC PCR of *E. coli* isolates from clinical sources ....................... 321
    7.5.1.4 A comparison: ERIC PCR of *E. coli* isolates from different sources (estuary, seafood and clinical) .................................................. 322
  7.5.2 Randomly amplified polymorphic DNA (RAPD) analysis of *E. coli* isolates ........................................................................................................ 323
    7.5.2.1 RAPD analysis using primer 1 ............................................................ 323
      7.5.2.1.1 RAPD of *E. coli* isolates from different sources using primer 1 ................................................................. 325
    7.5.2.2 RAPD analysis using primer 2 ............................................................ 326
      7.5.2.2.1 RAPD of *E. coli* isolates from different sources using primer 2 ................................................................. 327
  7.5.3 Restriction Fragment Length Polymorphism of *E. coli* isolates .......... 328
    7.5.3.1 RFLP using *EcoR I* ........................................................................... 328
      7.5.3.1.1 RFLP of *E. coli* isolates from Cochin estuary using *EcoR I* .................. 329
      7.5.3.1.2 RFLP of *E. coli* isolates from seafood sources using *EcoR I* .................. 330
      7.5.3.1.3 RFLP of *E. coli* isolates from clinical sources using *EcoR I* .................. 331
### Chapter 7

**7.5.3.1.4** A comparison: RFLP of *E. coli* isolates from different sources using *EcoR I* (estuary, seafood, and clinical) ........................................ 332

**7.5.3.2** RFLP of *E. coli* isolates using *Hind III* ........................................ 333

**7.5.3.2.1** RFLP of *E. coli* isolates from Cochin estuary using *Hind III* ........................................ 335

**7.5.3.2.2** RFLP of *E. coli* isolates from seafood sources using *Hind III* ........................................ 336

**7.5.3.2.3** RFLP of *E. coli* isolates from clinical sources using *Hind III* ........................................ 337

**7.5.3.2.4** A comparison: RFLP of *E. coli* isolates from different sources using *Hind III* (estuary, seafood, and clinical) ........................................ 339

### 7.6 Discussion

**7.6.1** ERIC PCR ................................................................................ 340

**7.6.1.1** ERIC PCR of *E. coli* isolates from Cochin estuary .................. 340

**7.6.1.2** ERIC PCR of *E. coli* isolates from seafood sources .......... 341

**7.6.1.3** ERIC PCR of *E. coli* isolates from clinical sources ............ 341

**7.6.1.4** A comparison: ERIC PCR of *E. coli* isolates from different sources ........................................................................... 342

**7.6.2** RAPD .......................................................... 343

**7.6.3** RFLP .................................................................................. 344

**7.6.3.1** RFLP of *E. coli* isolates from Cochin estuary .................. 344

**7.6.3.2** RFLP of *E. coli* isolates from seafood sources ............... 344

**7.6.3.3** RFLP of *E. coli* isolates from clinical sources ........... 344

**7.6.3.4** A comparison: RFLP of *E. coli* isolates from different sources ........................................................................... 345

### Chapter 8

**Characterization of a multidrug-resistant shiga toxin-producing ExPEC strain (CUSMBES11) .......... 347 - 363**

8.1 Introduction ...................................................................................... 347

8.2 Review of literature .......................................................... 348

8.3 Objectives .................................................................................. 351

8.4 Materials and Methods .......................................................... 351

8.4.1 Source and serotype of the *E. coli* ............................................ 351

8.4.2 Antibiotic susceptibility testing ............................................. 351

8.4.3 MAR indexing ....................................................................... 351

8.4.4 Isolation of DNA from *E. coli* ............................................... 353

8.4.5 Isolation of plasmid DNA from *E. coli* .................................. 353

8.4.6 Phylogenetic analysis .......................................................... 353
8.4.7 PCR amplification of 16S rRNA gene ......................................... 353
8.4.8 DNA sequencing ......................................................................... 353
8.4.9 RNA isolation .............................................................................. 354
8.4.10 RNA concentration ...................................................................... 354
8.4.11 cDNA synthesis .......................................................................... 354
8.4.12 Real time PCR ............................................................................. 355
8.4.13 Statistical analysis ....................................................................... 357

8.5 Results ................................................................................................. 357
8.5.1 Serotype ........................................................................................ 357
8.5.2 Antibiotic resistance ...................................................................... 357
8.5.3 Phylogenetic group ......................................................................... 358
8.5.4 Real time PCR ............................................................................... 358
8.5.5 Antibiotic resistance genes ............................................................ 359
8.5.6 House-keeping genes ..................................................................... 359
8.5.7 Virulence factor genes ................................................................... 359
8.5.8 Shiga toxin-producing genes ......................................................... 360

8.6 Discussion ........................................................................................... 360

Chapter 9

Summary and Conclusion .......................................................... 365 - 372
9.1 Summary ............................................................................................. 365
9.2 Conclusion ........................................................................................... 370

References .............................................................................................. 373 - 499
Appendices ............................................................................................. 501 – 571
Publications ............................................................................................ 573 – 580