MOLECULAR CHARACTERIZATION OF CLINICAL ISOLATES OF ENTEROPATHOGENIC *ESCHERICHIA COLI* FROM MIRI SARAWAK

IRENE LAH

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2004

MOLECULAR CHARACTERIZATION OF CLINICAL ISOLATES OF ENTEROPATHOGENIC *ESCHERICHIA COLI* FROM MIRI SARAWAK

By

IRENE LAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2004

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

MOLECULAR CHARACTERIZATION OF CLINICAL ISOLATES OF ENTEROPATHOGENIC *ESCHERICHIA COLI* FROM MIRI SARAWAK

By

IRENE LAH

September 2004

Chairman: Professor Son Radu, Ph.D

Faculty: Food Science and Technology

A total of thirty two strains of clinical enteropathogenic *Escherichia coli* (EPEC) isolated from Hospital Miri, Sarawak were examined and further characterized by various molecular techniques. These techniques include the plasmid profiling, antimicrobial resistance, resistance and virulence genes detection by multiplex PCR, RAPD, ERIC and PFGE genomic fingerprinting. All the strains studied were found to exhibit multiple antibiotics resistance patterns to twelve antibiotics [penicillin (100%), teicoplanin (100%), vancomycin (100%), bacitrasin (97%), methicillin (97%), erythromycin (69%), ampicillin (63%), cephalothin (47%), streptomycin (25%), chloramphenicol (16%), kanamycin (6%) and nalidixic acid (3%)] used. Thirteen EPEC isolates were shown to encode ampicillin resistance by means of the bla_{TEM} gene respectively, and none of the EPEC isolates showed the presence of the *sip*B/C, *cml*A/*tet*R and $bla_{\text{PSE-1}}$ genes. The plasmid profiles obtained ranged in size from 1.8 MDa to 57 MDa. Two types of specific primer encoding the Shiga-like Toxin gene, the *SLT*II (584 bp) gene and *SLT*I (348 bp) were utilized in the multiplex PCR assay. Analysis carried out demonstrated that all were positive for the presence of the *SLT*II and *SLT*I gene. Two EPEC isolates analysed by PCR were confirmed to be the O157:H7 serogroup as determined by agglutination tests with specific

antisera. Three 50% G+C contents 10-mer random primers, the Gen 1-50-02 (5'-CCAAACTGCT-3'), Gen 1-50-08 (5'-GAGATGACGA-3'), and Gen1-50-09 (5'-TCGCTATCTC-3') were chosen after screening through ten random primers. In PFGE technique carried out, two kinds of restriction enzymes, the *Spe*I (5'-A CTAGT-3') and *Xba*I (5'-T CTAGA-3') were used to check for the *in-situ* DNA digestion pattern due to their inherit advantages of the short sequence of these enzymes. Both the RAPD polymorphism pattern and PFGE profile obtained showed a significant discriminatory fingerprinting among the 32 isolates under studied. A respective dendrogram was constructed from the binary data matrix obtained from the RAPD, ERIC and PFGE fingerprints to compare the diversity relationship among the 32 isolates. All the dendrograms were constructed utilizing the RAPDistance software package based on the data retrieved from the presence or absence of banding pattern. All the three molecular techniques of RAPD-, ERIC-, and PFGE genotyping showed a significant correlation whereby the first 16 and the second 16 strains of EPEC used in this study showed a closer relationship in the respective cluster groups as shown in the constructed dendrograms. From the overall results obtained both the RAPD and ERIC analysis showed greater discriminatory power compared to the other phenotypic and molecular characterization techniques used in this study. Our results demonstrate that the antimicrobial resistance,presence of resistance and virulence genes, plasmid profiling, multiplex PCR, RAPD-PCR fingerprinting, ERIC and PFGE profiling methods are useful as a suitable analysis tools for a rapid and reliable molecular typing and identification of EPEC.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENCIRIAN SECARA MOLEKUL ISOLASI KLINIKAL ENTEROPATHOGENIC *ESCHERICHIA COLI* DARI MIRI, SARAWAK

Oleh

IRENE LAH

September 2004

Pengerusi: Profesor Son Radu, Ph.D

Fakulti: Sains Makanan dan Teknologi

Sejumlah tiga puluh dua strain klinikal enteropathogenic *Escherichia coli* (EPEC) yang diisolasikan dari Hospital Miri, Sarawak telah diuji dan dikenalpasti dengan beberapa teknik molekul. Teknik-teknik ini merangkumi penganalisaan profil plasmid, kerintangan antibiotik, pengesanan gen kerintangan dan gen virulence menggunakan multiplek PCR, genomik fingerprint RAPD, ERIC dan PFGE. Kesemua strain yang digunakan di dalam kajian ini didapati mempamerkan kepelbagaian corak antibiotik terhadap dua belas jenis antibiotik [penicillin (100%), teicoplanin (100%), vancomycin (100%), bacitrasin (97%), methicillin (97%), erythromycin (69%), ampicillin (63%), cephalothin (47%), streptomycin (25%), chloramphenicol (16%), kanamycin (6%) and nalidixic acid (3%)] yang digunakan. Tiga belas isolat EPEC enkod kerintangan ampicillin yang bermaksud gen bla_{TEM} dan tiada isolat EPEC yang menunjukkan kehadiran gen *sip*B/C, *cml*A/*tet*R dan $bla_{\text{PSE-1}}$. Profil plasmid yang diperolehi menunjukkan julat saiz antara 1.8 MDa ke 57 MDa. Dua jenis primer spesifik mengkod gen "Shiga-like Toxin", iaitu *SLT*II (584 bp) dan *SLT*I (348 bp) telah digunakan di dalam assai multiplek PCR. Analisis yang dijalankan mendapati bahawa kesemua strain menunjukkan kehadiran gen *SLT*II dan *SLT*I. Dua EPEC strain telah disahkan sebagai O157:H7 melalui

multiplek PCR dan ujikaji agglutination dengan spesifik antisera. Tiga primer rawak (10-mer) yang mengandungi kandungan G+C sebanyak 50%, iaitu primer Gen 1-50-02 (5'-CCAAACTGCT-3'), Gen 1-50-08 (5'-GAGATGACGA-3'), dan Gen1-50-09 (5'-TCGCTATCTC-3') telah dipilih selepas diuji secara rawak dengan 10 primer. Dalam teknik PFGE, dua jenis enzim pemotong, iaitu *Spe*I (5'-A CTAGT-3') dan *Xba*I (5'-T CTAGA-3') telah digunakan untuk diuji kepada corak. Kedua-dua corak polymorphism RAPD dan profile PFGE yang diperolehi menunjukkan diskriminatori signifikan fingerprint antara 32 isolat dalam kajian. Dendrogram masing-masing yang dibina daripada data binari matrik yang diperolehi dari fingerprint RAPD, ERIC dan PFGE untuk dibandingkan dengan kepelbagaian hubungan diantara 32 isolat. Kesemua dendrogram yang dibina menggunakan RAPDistance software package sesuai dengan data yang dicari daripada kehadiran dan ketidak hadiran corak band. Kesemua tiga teknik molekul iaitu genotipikan RAPD, ERIC dan PFGE menunjukkan korelasi signifikan dimana 16 strain yang pertama dan 16 strain yang kedua isolat EPEc yang digunakan di dalam kajian ini menunjukkan hungan yang rapat antara satu sama lain bagi setiap kumpulan cluster yang ditunjukkan di dalam dendrogram yang telah dibina. Daripada keselurahan keputusan yang diperolehi, kedua-dua penganalisis RAPD dan ERIC menunjukkan kuasa diskriminatori yang terbaik berbanding dengan fenotip dan pengenalpastian teknik molekul yang lain yang digunakan di dalam kajian ini. Keputusan menunjukkan bahawa kerintangan antibiotik dan gen, profil plasmid, multiplek PCR, fingerprint RAPD PCR, kaedah profil ERIC dan PFGE berfaedah sebagai analisis yang sesuai untuk rapid dan jenis molekul dan identifikasi EPEC.

LIST OF TABLES

LIST OF FIGURES

Figure ***Page***

LIST OF ABBREVIATIONS

A	adenine or adenosine
AP-PCR	arbitrarily primed-polymerase chain reaction
ATCC	American type culture collection
ATP	adenosine triphosphate
Am	ampicillin
B	bacitracin
bp	basepair
BSA	bovine serum albumin
C	chloramphenicol
Car	carbenicillin
Cf	cephalothin
ccc	covalently closed circular
CN	gentamicin
cm	centimetre
Da	dalton (the unit of molecular mass)
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dH_2O	Distilled water
DI	discriminatory index
DNA	Deoxyribonucleic acid

dTTP	Deoxythymidine triphosphate
E	erythromycin
EAEC	enteroaggregative *Escherichia coli*
EAF	EPEC adherence factor
E. coli	*Escherichia coli*
e.g.	For example
EDTA	Ethylenediamine tetraacetic acid
EHEC	enterohemorrhagic *Escherichia coli*
EIEC	enteroinvasive *Escherichia coli*
EPEC	enteropathogenic *Escherichia coli*
ETEC	enterotoxigenic *Escherichia coli*
EtBr	ethidium bromide
gm	gram
g	gravity
G	Guanine
GM	gentamicin
GTP	Guanosine triphosphate
H_2O	Water
HCl	Hydrochloric acid
i.e.	that is
ID	Identification number
K	kanamycin
KAc	potassium acetate

kb	Kilobase pair (number of bases in thousands)
Kda	kiloDalton
kg	kilogram
l	litre
LB	Luria-Bertani
M	Molar, or molarity, moles of solute per liter of slution
mA	miliamphere
MAR	Multiple Antibiotic Resistance
MDa	megadalton
Met	methicillin
mg	miligram
MHA	mueller Hinton agar
min	Minutes
ml	Mililiter
mm	millimeter
mM	Millimolar
µg	Microgram
µl	Microliter
mol	mole
Nal	nalidixic acid
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
ng	Nanogram

P	penicillin
%	Percent
R	resistant
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	ribonucleic acid
Rnase	ribonuclease
rpm	revolution per minute
S	sensitive
S	streptomycin
sdH_2O	Sterile distilled water
SDS	sodium dodecyl sulphate
TAE	Tris acetate EDTA electrophoresis buffer
Taq	*Thermus aquaticus* DNA (polymerase)
TBE	Tris borate EDTA electrophoresis buffer
TE	tris-EDTA
TEC	teicoplanin
Tris	tris (hydroxymethyl) methylamine
UV	ultraviolet
V	Volts
Van	vancomycin
w	weight
^oC	degree Celsius

ACKNOWLEDGEMENTS

My deepest gratitude goes to my supervisor, Professor Dr. Son Radu for trusting me and giving me the opportunity to do my MSc. I treasured and appreciate his patience, guidance, advices and encouragement through out my study years. I learn a lot from him.

My appreciation also goes to Associate Professor Dr. Raha Abdul Rahim and Dr. Clemente Michael Wong Vui Ling for their support and cooperation. They have been very helpful.

Not forgetting my family (dad, mum, Amie, Flo, Urei, Unen, Abo' Uk, and Puyang) for their unending love, prayers and encouragement. Words could not express my gratitude to all of you. Special thanks goes to all my aunts, uncles, grandpa, grandma and cousins for their love and prayers.

Also, I thank my lab friends (Wai Ling, Les, Gwen, Kqueen, Sam, Jurin, Kak Zaleha, Ibu Endang, Sushil, John Lawrence, Yousr, Kak Zila, Yin Sze, Bell, Liha, Tung, and Daniel) for helping me in my work and patiently teaching me so many things. Thank you for your friendship, it means a lot to me.

Also I would like to express thanks to all my housemates (Chris, Thy, Sera, Emmy, Cath, Gina, Arish, Ah Lai and Charlyn), our pastor (Pr. Kenny Tham and family; Pr. Jung and family), our cell group members(Mim, Mine, Elly, Emma, and Sue) and brothers and sisters in Christ SIB Serdang for their support, help and prayers. I am so bless to have all of you by my side….. All glory, honor, praise and thanksgiving be unto the Lord.

Last but not least, my most heartfelt appreciation goes to Chris Gala Innue (my one and only sweetheart) – thanks for your encouragement and prayers as well as mentally support.

CHAPTER I

GENERAL INTRODUCTION

Escherichia coli is the predominant facultative anaerobic in the human colonic flora. It usually remains harmlessly confined to the intestinal lumen; however, in the debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, even *E. coli* strains of normal flora can cause infection. Three general clinical syndromes result from infection with pathogenic *E. coli* strain are (i) urinary tract infection: (ii) sepsis/meningitis; and (iii) enteric/diarrhoeal disease (Nataro and Kaper, 1998). Moreover, even the most robust members of our species may be susceptible to infection by one of several highly adapted *E.coli* clones which together have evolved the ability to cause a broad spectrum of human diseases. Infections due to pathogenic *E. coli* maybe limited to the mucosal surfaces or can disseminate throughout the body. Three general paradigms have been described by which *E. coli* may cause diarrhea are (i) enterotoxin production (ETEC and EAEC), (ii) invasion (EIEC), and/or (iii) intimate adherence with membrane signalling (EPEC and EHEC). However, the interaction of the organisms with the intestinal mucosa is specific for each category. The versatility of the *E. coli* genome is conferred mainly by two genetic configurations: virulence-related plasmids and chromosomal pathogenicity islands. All six categories of diarrheagenic *E. coli* have been shown to carry at least one virulence-related property upon a plasmid. EIEC, EHEC, EAEC, and EPEC strains typically harbor highly conserved plasmid families, each encoding multiple virulence factors (Hales et al., 1992; Nataro and Kaper, 1987; Wood et al., 1986). McDaniel and Kaper have shown recently that the chromosomal virulence genes of EPEC and EHEC are organized as a cluster referred to as a pathogenicity island (McDaniel et al., 1995; McDaniel and Kaper, 1997).

Such islands have been described for uropathogenic *E. coli* strains (Donnenberg and Welch, 1996) and systemic *E. coli* strains (Bloch and Rode, 1996) as well and may represent a common way in which the genomes of pathogenic and nonpathogenic *E. coli* strains diverge genetically.

Fecal-oral and food borne transmission of *E.coli* are well documented (Nataro and Kaper, 1998). As with other diarrheagenic *E. coli*, transmission of enteropathogenic *E. coli* (EPEC) is fecal-oral, with contaminated hands, contaminated food, or contaminated fomites serving as vehicles. In adults outbreak, waterborne and foodborne transmissions have been reported, but no particular type of food has been implicated as more likely to serve as a source of infection (Levine and Edelman, 1984). The most notable feature of type epidemiology of disease due to EPEC is the striking age distribution seen in persons infected with this pathogen. EPEC infection is primarily a disease of younger than 2 years. Illness caused by EPEC is often clinically acute severe diarrhea. However, EPEC can cause diarrhea in an adult if the bacterial inoculums is high enough (Nataro and Kaper, 1998).

Pathogenic human and animal *E. coli* resistant to many classes of antimicrobial agents have been reported worldwide (Bradford *et al*., 1997; Winolur *et al*., 2001). These multi resistant pathogens present an important challenge to achieving effective therapy. Antimicrobial resistance in commensal strains of *E. coli*, however, may also play an important role in the ecology of resistance clinical infectious diseases. Transmission of resistance genes from normally nonpathogenic species to other virulent organism within the animal or humans interinal tact may be an important mechanism for acquiring clinically significant antimicrobial-resistant organisms. *E. coli* may serve as an important reservoir for these transmissible resistances, since it

is clear that this organism has developed a number of elaborate mechanisms for acquiring and disseminating plasmids, transposons, phage, and other genetic determinants (Neidhardt, 1996).

The resistance phenotypes may arise from many different genetic determents and each determinate may present specific epidemiological features. Therefore, the assessment of the resistance situation at the genetic level would be an important asset in the understanding and control of antimicrobial resistance in general. Antibiotic resistance genes found in nature are organized in gene cassettes and may be included in intergrons. Intergrons may be transferred between bacteria and higher cells at high frequencies. Disc diffusion is the method always used in the laboratory to determine antibiotic resistance phenotypes. The resistant of isolates toward antibiotic is shown with the clear zone formed around the antibiotic-containing disc.

The pathogenicity of EPEC is encoded by plasmids, but bacterial chromosome also brings the effect of the pathogenesis for diarrhea. The molecular weight of plasmids can indicate the characteristics of conjugation in transferring the resistant genes. The high copy number and low molecular weight plasmids can indicate the efficiency in conjugation of genetic information. Normally, plasmids bring drug-resistant genes (R plasmids) toward certain antibiotics presented in the environment so that bacterial cells can survive in the environment.

In recent years, the use of molecular “fingerprinting” methods has become standard practice in microbiology for evaluating the epidemiology of infectious diseases, investigating suspected outbreaks of bacterial infection, and trying bacterial (Mickelsen, 1997). Pulsed-field gel electrophoresis (PFGE) allows the generation of simplified chromosomal restriction fragment

patterns without having to resort to probe hybridization methods. In this method, restriction enzymes that infrequently cut DNA are used for generating large fragments of chromosomal DNA, which are then separated by special electrophoresis (Swaminatham and Matar, 1993). PFGE has been applied to sub typing of several Gram-positive and Gram-negative bacteria. It is not widely used in epidemiological surveillance and common interpretation schemes have been published (Tenovar *et al.,* 1997).

Another widely used method is Polymerase chain reaction (PCR). In PCR, a pair of primers (20-40 bases) is used for selective amplification and detection of a certain DNA sequence in a target orgasm. PCR primers have successfully been developed for all categories of dirrheagenic *E. coli.* PCR can be used in both diagnosing and typing *E.coli* strains. Advantages of PCR include high sensitivity, specificity and appropriate rapidity in the detection of target DNA templates. In diagnostics, PCR is commonly used for detecting different virulence associated genes of *E. coli*, such as toxin and adherence associated genes. PCR is also widely used in sub typing by doing virulence geneproflie for different diarrheagenic *E. coli* strains. The RAPD, a PCR based methods has been used as a sensitive and efficient method for distinguishing and study of genetic relatedness among the isolates. A pair of short primers is bind randomly to the DNA sequence and amplified into bands. The same RAPD pattern shown will indicate the higher possibility of the isolates was derived from the same serogroups. Other techniques such as enterobacterial repetitive intergenic consensus (ERIC) PCR (Gison *et al.,* 1984; Hulton *et al.,* 2001; de Moura *et al.,* 2001). The study conducted by Versalovic *et al*. (1991) had demonstrated that the ERIC-like sequences are present in many diverse eubacterial

species, and also these sequences, can be utilized as efficient prier binding sites in the PCR reaction to produce fingerprints of different bacterial genomes.

The latex agglutination test (Verotox-F assay, denka Seyken, Tokio, Japan) for detection of toxins produced by Shiga Toxin-producing *E. coli* (STEC) has been found 100% sensitive and 100% specific in comparison with the classical Vero cell assay (Karmali *et al*., 1999). Immunomagnetic separation with magnetic beads coated with antibody against *E.coli* O157 have been found more sensitive than direct culture of these strains (Chapman and Siddons, 1996).

All these new molecular typing methods have allowed highly discriminant genotyping, and are useful tools for demonstrating that isolates from different sources are identical, closely related or not related at all (Mickelsen, 1997). The molecular biotyping techniques are now well accepted as one of the most important and useful differentiation tool for studying the molecular characteristics of isolates, an understanding of genetic variability in *E. coli* is important for studies of the taxanomy, epidemiology and pathogenicity of this species.

Objective of Study

The objectives of this study are:

1. To detect the presence of the specific gene sequence responsible for the production of the 'shiga like-toxin' by multiplex-polymerase chain reaction (multiplex-PCR) technique.

2. To determine the antibiotic susceptibility patterns and plasmid profiles among EPEC isolates.
3. To carry out DNA-based genotyping techniques for the EPEC isolated using randomly amplified polymorphic DNA analysis (RAPD) ERIC-PRC and pulsed-field gel electrophoresis (PFGE).
4. To compare the discriminatory power of antibiotic resistance patterns, plasmid profiles, RAPD-, ERIC-PCR and PFGE analysis for typing the EPEC isolates.

CHAPTER II

LITERATURE REVIEW

Enteropathogenic *Escherichia coli* (EPEC)

Enteropathogenic *Escherichia coli* (EPEC) is an important category of diarrheagenic *E. coli* which has been linked to infant diarrhea in the developing world. Once defined solely on the basis of O and H serotypes, EPEC is now defined on the basis of pathogenetic characteristics.

Since the 17th century, diarrhea had become the real death cause of children during summer. So, it had been termed as "summer diarrhea" (Creighton, 1975). After one century, Albert *et al*. (1995) reported again that *E. coli* infections peaked during dry summer months, from February to May in Bangladesh, a tropical country. Enteropathogenic *Escherichia coli* (EPEC) is a leading cause of infantile diarrhea in developing countries. In industrialized countries, the frequency of these organisms has decreased, but they continue to be an important cause of diarrhea (Nataro *et al*., 1998). The central mechanism of EPEC pathogenesis is a lesion called attaching and effacing (A/E), which is characterized by microvilli destruction, intimate adherence of bacteria to the intestinal epithelium, pedestal formation, and aggregation of polarized actin and other elements of the cytoskeleton at sites of bacterial attachment. The fluorescent actin staining test allows the identification of strains that produce A/E lesions, through detection of aggregated actin filaments beneath the attached bacteria (Knutton *et al*., 1989). Ability to produce A/E lesions has also been detected in strains of Shiga toxin-producing

E. coli (enterohemorrhagic *E. coli* [EHEC] and in strains of other bacterial species (Nataro *et al*., 1998). The emergence and rise in frequency of atypical EPEC strains may have origins similar to those that led to the emergence and increase in frequency of O157:H7 and other STEC serotypes (Griffin, 1998).

Enteropathogenic *Escherichia coli* (EPEC) has been recognized as a common cause of watery diarrhea in children (Moyenuddin *et al*., 1989; Sethi and Khuffash, 1989). A study conducted in Malaysia (Jegathesan *et al*., 1975) showed that 9% of the diarrheal cases in children under 10 years of age in Malaysia were due to EPEC. The reason(s) for the relative resistance of adults and older children is not known, but loss of specific receptors with age is one possibility. However, EPEC can cause diarrheal in an adult if the bacterial inoculum is high enough. The infectious dose in naturally transmitted infection in infants is not known, but it is presumed to be much lower than with adults (Nataro and Kaper, 1998). EPEC strains are an important cause of disease in all settings of nosocomial outbreaks, outpatient clinics, patients admitted to hospitals, community-based longitudinal studies, and urban and rural settings (Nataro and Kaper, 1998).

The most notable feature of the epidemiology of disease due to EPEC is the striking age distribution seen in persons infected with this pathogen. EPEC infection is primarily a disease of infants younger than 2 years. As reviewed by Levine and Edelman (Levine and Edelman, 1984), numerous case-control studies in many countries have shown a strong correlation of isolation of EPEC from infants with diarrhea compared to healthy infants. The correlation is strongest with infants younger than 6 months. In children older than 2 years, EPEC can be isolated from healthy and sick individuals, but a statistically significant correlation with disease is usually not found.

Another virulence property that has been associated with EPEC is the production of verotoxin (*VT*), also known as Shiga-like toxin. Although strains from outbreaks usually did not produce *VT*, some strains, in particular of serogroups O26 and O111, from sporadic cases of diarrhea or hemolytic-uremic syndrome have been shown to possess this property (Smith *et al.,* 1990; Scotland *et al.,* 1990; Caprioll *et al.,* 1992; Willshaw *et al.,* 1992; Caprioll *et al.,* 1994) and are currently classified as EHEC. Therefore, although the mechanism by which EPEC strains evoke a fluid response in the intestine of the human host is still unclear, many virulence-associated factors have been identified in this group of organisms, and some of them have been proposed as possible markers for EPEC identification, in addition to or as replacement for the O serogrouping (Levine *et al*., 1988; Knutton *et al*., 1989). However, recent studies suggest that not all the virulence factors are evenly distributed in the wild-type EPEC strains circulating in different geographical areas (Levine *et al*., 1988; Smith *et al*., 1990; Knutton *et al*., 1991; Scotland *et al*., 1991; Morelli *et al*., 1994).

The Shiga toxin (*stx*) genes are part of the genome of temperate lambdoid phages, which are integrated in the chromosome of the bacterial host. At present, the ability to produce Stx has been assigned to more than 200 *E. coli* serotypes, which have been isolated from patients, healthy humans (Nataro and Kaper, 1998), animals, food (Doyle and Schoeni, 1987), and water (Muniesa and Jofre, 1998). Stx production was observed also in other members of the *Enterobacteriaceae*, including *Citrobacter freundii* (Schmidt et al., 1993; Tschape et al., 1995) *Enterobacter cloacae* (Paton and Paton, 1996), *Shigella sonnei* (Strauch et al., 2001), and *Shigella dysenteriae* I (Strockbine et al., 1988).