شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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جامعة عين شمس

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Molecular Characterization of shiga-toxin producing *Escherichia coli* in Milk

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For the degree of M. V. Sc.
(Bacteriology, Immunology and Mycology)

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Abstract

Recently, the incidence of food-borne diseases caused by Shiga toxin-producing *Escherichia coli* (STEC) has increased around the world. **Aim:** In this study, we have examined the methodologies and molecular characterization techniques for assessing the phenotypic, genotypic and characteristics of STEC O157 and non-O157 from milk samples from different governorates in Egypt. **Method:** milk samples were collected from different governorates in Egypt, Culture and isolation methods, including selective enrichment and differential plating that enabled the effective recovery of STEC have been performed. Following recovery, immunological serotyping of somatic surface antigens (O-antigens) was employed for the classification of the STEC isolates. Molecular genotyping methods, including polymerase chain reaction (PCR), and partial genome sequencing have been performed.

Results: In the present study, out of 33/158 samples were positive for *Stx*2 gene (20.88), 1 sample was positive for *Stx*1 and *Stx*2 and 10 isolates were positive for *eae* gene**.** Also in this study, 7 serogroups were found between the isolates of *E. coli* (O157, O26, O111, O78, O125, O158, O127). O157 (5.06%) and O26 (3.79%) were the most frequently identified serogroups.

Conclusion: High prevalance of *Stx*2 in collected milk samples and high prevalence of O157, O26 strains and other important serogroups will provide a better understanding of risks associated with STEC and will aid in the development of efficient and targeted intervention strategies.

Keywords:
Shiga toxin producing *Escherichia coli*, Raw milk, Molecular Characterization, Serotyping.

Dedication

This thesis is dedicated to my dearest father who helped me to complete this work and throughout my life.
He did not live to see my academic achievement.
God bless his soul.

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Aya Gomaa

Contents

Abbreviations

Abbreviation	Full Name
ABU	Asymptomatic Bacteriuria
BLAST	Basic Local Alignment Search Tool
Bp	Base pair
CFU	Colony Forming Units
Ch:I	Chloroform:Isoamyl
CLSI	The Clinical and Laboratory Standards Institute
CTAB	Cetyl Trimethyl Ammonium Bromide
DAEC	Diffusely Adherent *E. coli*
DEC	Diarrheagenic *E. coli*
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
eae	Intimin gene
EAEC	Enteroaggregative *E. coli*
ECDC	European Centre for Disease prevention and Control
EFSA	European Food Safety Authority
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EMB	Eosin Methylene Blue
EPEC	Enteropathogenic *E. coli*

ETEC	Enterotoxigenic *E. coli*
ExPEC	extraintestinal pathogenic *E. coli*
HACCP	Hazard Analysis and Critical Control Point
HC	Hemorrhagic Colitis
hlyA	Hemolysin gene
HUS	Hemolytic Uremic Syndrome
IMS	Immunomagnetic Separation
Kb	Kilo-base
LEE	Locus of Enterocyte Effacement
LPS	Lipopolysaccharide
Mb	Megabase
MDR	Multiple Drug Resistance
Mg	Microgram
mg/L	Milligram per liter
MIC	Minimum Inhibitory Concentrations
Ml	Milliliter
MLEE	Multi-locus Enzyme Electrophoresis
MLVA	Multilocus VNTR analysis
MNEC	Meningitis-associated *E. coli*
NARMS	National Antimicrobial Resistance Monitoring System
NCBI	National Centre for Biotechnology Information

NM	Non-motile
NTEC	Necrotoxigenic *E. coli*
PAI or PI	Pathogenicity Islands
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
pH	Hydrogen ion concentration
qPCR	Real-time quantitative PCR
RAPD	Random Amplified Polymorphic DNA
STEC	Shiga toxin-producing *Escherichia coli*
***Stx* 2**	Shiga Toxin 2 gene
***Stx*1**	Shiga Toxin 1 gene
TAE	Tris-acetate-EDTA
tRNA	Transfer RiboNucleic Acid
TSB	Tryptone Soya Broth
UPEC	Uropathogenic *E. coli*
USA	United States
USDA	United states Department of Agriculture
UTIs	Urinary Tract Infections
VFs	Virulence Factors
VNTR	Variable Number Tandem Repeat
VTEC	Verocytotoxigenic *E. coli*

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