Isolation of Shiga-toxin producing *E. coli* (STEC) from fresh food and their natural environment

Thesis Submitted for
A Ph.D. Degree in Science (Microbiology)

By

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B.Sc. in Microbiology/Chemistry (2004)

M.Sc. in Microbiology-Ain Shams University (2012)

Microbiology Department,
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2017

Approval Sheet

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List of abbreviations and symbols

STEC	:	Shiga-toxin producing *Escherichia coli*
VTEC	:	Verocytotoxin producing *Escherichia coli*
PCR	:	Polymerase Chain Reaction
m-TSB	:	Modified Tryptone- Soy Broth
BPW	:	Buffered Peptone Water
CT-SMAC	:	Cefixime Tellurite Sorbitol MacConkey agar
TBX	:	Tryptone Bile X-glucuronide medium
ECDC	:	European Centre for Disease Control and Prevention
HUS	:	Hemolytic Uremic Syndrome
EFSA	:	European Food Safety Authority
PAI	:	Pathogenicity Islands
CDC	:	Centers for Disease Control
UPEC	:	Uropathogenic *E. coli*
ETEC	:	Enterotoxigenic *E. coli*
EPEC	:	Enteropathogenic *E. coli*
EHEC	:	Enterohemorrhagic *E. coli*
DAEC	:	Diffuse adhering *E. coli*
EAEC	:	Enteroaggregative *E. coli*
EIEC	:	Enteroinvasive *E. coli*
HC	:	Haemorrhagic Colitis
AE	:	Attachment and Effacing lesions
FDA	:	Food and Drug Administration
USDA	:	United States Department of Agriculture
APHA	:	American Public Health Association
ESBL	:	Extended-Spectrum – β lactamase
NCCLS	:	National Committee for Clinical and Lab Standards
MIC	:	Minimum Inhibitory Concertation
TEM	:	Transmission Electron Microscope
bla	:	beta-lactamase or β-lactamase
MIC	:	Minimum Inhibitory Concentration
MRD	:	Multi Drug Resistant
$bla_{\text{U-CTX-}\mathbf{M}}$	:	Universal CTX-M
PG	:	Penicillin G
TS	:	trimethoprim + sulfamethaxazole
T	:	Tetracyclin
IMI	:	Imipenem
VA	:	Vancomycin
CTX	:	Cefotaxim
NOR	:	Norofloxacin
GM	:	Gentamycin
CAZ	:	Ceftazidime
CPM	:	Cefepime

Summary

Shiga-toxin producing *E. coli* (STEC) is a pathogen that causes diseases in humans, ranging from non-bloody diarrhea to severe illnesses as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Ruminants are regarded as the natural reservoir for Shiga toxin-producing *Escherichia coli* (STEC). Monitoring of ruminants and environmental samples (such as irrigation water and soil) is essential to evaluate the risk factor associated with STEC infection in humans.

This study highlights the occurrence of different types of O157 STEC and non-O157 STEC in animals such as cattle, sheep, goat and calves; environmental samples as irrigation water, soil and food samples of plant and animal origins.

A total of 642 samples were investigated directly for Shiga-toxin encoded genes by Real Time PCR method using *stx* and *eae* specific probes. After enrichment of the samples isolation of STEC on selective media was carried out. Suspected colonies were tested for shiga-toxin by Real Time PCR.

Results indicated that out of the642 samples only 36 isolates were positive to one or more of the previously mentioned genes. Out of 120 fecal samples from different ruminants, 16.7% were positive for Shiga toxin. The highest percent of positive STEC samples was recorded in buffalos (30%) were positive,

while cows (15%); sheep (20%); goats (10%) and calves (20%). These results indicated the high prevalence of STEC in buffalos than cows and sheep. Irrigation water samples showed (8.3%) positive STEC. All the drinking water samples (25 samples) were negative.

Investigation of food samples indicated that (8%) of minced beef samples; (10%) chicken fillets and (4%) processed meat (4 %) in raw milk were found to be contaminated with STEC. However STEC was not isolated from the plants during this study.

Serotyping of the isolates revealed that serotype O157 was the most predominant serotype in water samples, while other various serotypes and non-O157 such as O78, O55, O26, O1 and O126 were distributed in the rest of the isolates recovered from the other sources. It was noted that the presence of non-O157 was greater than O157 in fecal samples and Milk.

STEC were not isolated from human fecal samples and screening using real time PCR didn't give any amplification for the *stx1* or *stx2* genes. However, *eae* gene was isolated from various samples suggesting the presence of a pathogenic *E. coli* other than STEC which may be enteropathogenic *E. coli*.

The German strain of the year 2011 (*E. coli* O104:H4-2011) was not isolated or detected in this study suggesting that

this strain is not found in our environment and that the causative agent of the 2011 outbreak was not originated from Egypt.

Antibiotic susceptibility of STEC isolates indicated that all the isolates were resistant to penicilliG, vancomycin, while most of them were resistant to the tetracyclin. All isolates were sensitive to the imipenem, gentamycin and norofloxacin. Isolates recovered from animal fecal samples and food samples were the most resistant isolates, some were resistant to 6 antibiotics.

Twelve isolates were selected from different samples for studying the distribution of shiga-toxin subtypes; it was noticed that the most frequent subtype was *stx2* type (c) followed by *stx2* subtype (d), and the least to be recorded was Subtype (a). A combination of *stx2* subtypes was recorded in some isolates.

Detection of β lactamase resistance genes (bla_{CTX}, bla_{SHV} *and* bla_{TEM}) was also studied; this study confirms the prevalence of ESBL-producing bacteria of STEC group and demonstrates the spread of genes encoding ESBLs among STEC isolated from animals and/or the environment.

Silver nano-particles are useful for medical applications due to their strong antibacterial activity. In this study, silver nano-particles were synthesized using specific chemical method and it was characterized by UV-vis spectroscopy and transmission

electron microscope (TEM). Spherical nano-silver proved to have potential against *E. coli* and STEC.

Conclusion and Recommendation

- This study provides evidence that Shiga-toxin producing *E. coli* of zoonotic origin can contaminate the environment such as irrigation water as a result of the discharge of ruminants.
- Cattle, sheep and goat are possible sources of the transmission of this pathogen to the environment.
- The lack of enforcement of good hygiene practice and good manufacturing practice may ease the transmission of STEC to contaminate products.
- Raw milk and unpasteurized milk may pose a possible source for the contamination by STEC.
- Real Time PCR is a power tool for the detection of STEC in food and environmental sample.
- New pathogenic strains may emerge as a result of exchange of genetic materials.
- The German strain *E. coli* O104:H4-2011 is not found in our environment and also suggesting that the causative agent of the 2011 outbreak was not originated from Egypt.
- STEC strains isolated from food, environment and animals have developed antibiotic resistance that highlight the importance of gene transfer in the distribution of antibiotic resistance in the environment.

Recommendation:-

1- Using of Real Time PCR method as an accurate and very fast diagnostic tool for the detection of food born pathogen such as Shiga-toxin producing *E. coli*as this group of pathogens are very similar in

theirmorphological and biochemical characteristics with other groups of pathogenic *E. coli*.

2- Antibiotics should be used with a great concern in both animal and human to avoid the emergence and spread of antibiotic resistance between pathogenic bacteria.
3- Attention should be taken to the ruminants which are considered as the main reservoir of STEC; other group of animals such as sheep and goat are considered to be a possible source of the transmission of this group of pathogen.
4- Vaccination against STEC may be considered a possible solution of this problem and to reduce its prevalence in the cattle population.

5- Using filters containing Nano-Silver particles can be used as a preventive tool for the purification of irrigation water from this pathogen.
6- Food should be handled and cooked in a proper way; milk should be pasteurized and never consumed raw to avoid the infection of human.
7- Good hygienic practice and good manufacturing practice are very important for the processing of meat products
8- Further researches are needed to develop a better culture media and diagnostic tools to discriminate between different pathogenic groups of *E. coli*.
9- Future studies are needed to study the role of mobile genetic elementsin the transfer of resistance genes and virulence genes.