Cairo University
Faculty of Veterinary Medicine
Department of Zoonoses

Risks of Zoonotic Bacterial Pathogens Associated With Water Use in Egypt

Thesis Presented by

Esraa Abdul Majeed Mohammed
(B.V.Sc., 2008)
(M.V.Sc., 2012)
Fac. Vet. Med., Cairo University

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(Zoonoses)

Under The Supervision Of

Prof. Dr.Osman M. Hamed
Professor of Zoonoses
Fac. Vet. Med., Cairo University

Prof. Dr. Wafaa W. M. Reda
Professor of Zoonoses
Fac. Vet. Med., Cairo University

Dr. Khaled Abd El Aziz Abd El- Moein
Assistant Professor of Zoonoses
Fac. Vet. Med., Cairo University

Prof. DrMohga F. M. Badawi
Professor of Animal Hygiene
Fac. Vet. Med., Cairo University

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Cairo University
Faculty of veterinary medicine
Zoonoses Department

Supervision sheet

Prof. Dr. Osman M. Hamed
Professor of Zoonoses
Fac. Vet. Med., Cairo University

Prof. Dr. Wafaa W. M. Red
Professor of Zoonoses
Fac. Vet. Med., Cairo University

Dr. Khaled Abd El Aziz Abd El- Moein
Assistant Professor of Zoonoses
Fac. Vet. Med., Cairo University

Prof. Dr. Mohga F. M. Badawi
Professor of Animal Hygiene
Fac. Vet. Med., Cairo University

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Faculty of Veterinary Medicine.
Department of Zoonoses.

-Name : Esraa Abdul Mageed Mohammed.
-Date of birth : 17/1/1987.
-Nationality : Egyptian.
-Degree : PhD degree.
-Specialization: Zoonoses.
-Title of thesis: *Risks of zoonotic bacterial pathogens associated with water use in Egypt*
-Under supervision of:
Prof. Dr. Osman Mohamed Hamed
Prof. Dr. Wafaa Waheid Mohamed Reda
Dr. Khaled Abdel- Aziz Abdel- Moein
Prof. Dr. Mohga Fouad Mahmoud Badawi

Abstract

The current study was carried out to investigate the public health implications associated with different irrigation waters. For this purpose, twenty water samples (5groundwater, 11surface water and 4wastewater) were collected from irrigation streams in different localities in Egypt. In addition, 162 tomatoes, 89 animals and 60 farm workers samples from fresh produce farms were enrolled in the current study. Isolation of *Escherichia coli* and *Pseudomonas aeruginosa* from water samples was performed using membrane filtration technique then plating was done on selective media, while tomato and animal samples were enriched in Tryptic Soya Broth before streaked on such selective media. Isolates were identified through colonial characters; Gram's stain and biochemical reactions. Multiplex PCR was performed to identify different *E.coli* genotypes while conventional PCR was performed to detect Exotoxin A gene (*toxA)* from *P. aeruginosa.* The results revealed that the microbial quality of irrigation water was varied according to its source, being groundwater usually of good microbial quality whereas wastewater is the poorest one. Highest prevalence of *E.coli* and *P. aeruginosa* was recorded in tomatoes irrigated with surface water, in contrast *E.coli* was not found in tomatoes irrigated with groundwater .Verotoxigenic *E.coli* (VTEC) was the most frequently genotype recovered from *E. coli* isolates (74.3%). *Vt2* gene was the predominate gene among VTEC tomato isolates. *P. aeruginosa* isolates were positive to Exotoxin A gene *(toxA)* with percentage (57.3%). Sequencing and phylogenetic tree of 3 *P. aeruginosa* sequences proved that the water and tomato sequences were more identical to each other recording (98%) than cattle sequence. In conclusion, the current study underscored the potential role of irrigation water in the transmission of water-borne pathogens to both man and animals through direct/or indirect ways via contamination of fresh produce a matter which carries a great public health implication.

(Key words: irrigation water- tomato-*Escherichia coli- Pseudomonas aeruginosa*- -public health).

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List of abbreviation

AHRI	Animal health research institute
AK	Amikacin
AMC	Amoxicillin/clavulanate
BLAST	Basic Local Alignment Search Tool
CAZ	Ceftazidime
CIP	Ciprofloxacin
CN	Gentamicin
C-N	Ceterimide -Sodium nalidixate
EAEC	Enteroaggregative *Escherichia coli*
EHEC	Enteroheamorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EMB	Ethylene methylene blue
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FEP	Cefipime
Gb3	Globotriaosylceramide
HUS	Hemolytic uremic syndrome
IMP	Impenem
NCCLS	National Committee for Clinical Laboratory Standards
NCBI	National center of biotechnology information
PRL	Pipracillin
STEC	Shigatoxegenic *Escherichia coli*
TOB	Tobramycin
***tox* A**	Exotoxin A gene
TSB	Tryptic soya broth
USEPA	US Environmental Protection Agency
VT	Verotoxin
VTEC	Verotoxegenic *Escherichia coli*

Introduction

Water is required for many agricultural operations, from livestock watering and dairy shed cleaning, to crop irrigation, and even as an ingredient in food processing **(Pepper *et al.*, 2006)**.

Irrigation is a necessary system to many farming operations, however, contaminated irrigation water has the potential to further contaminate a large portion of crops that it is used to irrigate. Natural sources of water for irrigation include lakes, rivers, collected rainwater and groundwater. The potential for microbial contamination of these water sources varies significantly depending on variety of factors **(Suslow*et al.*, 2003).** Wastewater - or the so called greywater which is usually referring to domestic wastewater - has always been used for irrigation of agricultural crops. The irrigation water quality is of international importance because products exposed to microbiologically contaminated water may put consumers at risk both locally and internationally.

In Egypt, agriculture consumes the largest amount of the available water, with its share exceeding 85% of the total demand for water. The water sector in Egypt is facing many challenges including water scarcity and deterioration of water quality because of population increase and lack of financial resources**(Abdel Kader and Abdel Rassoul, 2010).**Conventional water resources in Egypt are limited to the Nile River; underground water in the deserts and Sinai, and precipitation. Each resource has its limitation on use. These limitations were related to quantity, quality, space, time, and/or use cost **(El**

Gamal *et al.*, 2005). However, Groundwater, surface water and wastewater are commonly used for irrigation in Egypt.

Fresh produce (fresh fruits and vegetables) consumption has been increasing worldwide for several decades **(Betts 2014).** Microbiologically, however, there can be some challenges in the production of safe fresh produce **(Betts 2014),** which has been accompanied by a rise in the number of produce-associated foodborne disease outbreaks. Based upon EU Zoonoses Monitoring data from 2007 to 2011, Foods of Non-Animal Origin (FoNAO) were associated with 10% of outbreaks, 26% of cases, 35% of hospitalizations, and 46% of deaths **(EFSA 2012).** Several potential sources of contamination during the production of fresh produce have been proposed including animal fertilizers, irrigation water and farm workers **(Grant *et al.*, 2011)**. The difficulty of tracing the original sources of contamination still has posed challenges to public health. However, outbreak investigations suggest that irrigation water is likely the most significant risk factor in horticultural production systems **(Cooley *et al.*, 2013).**

Therefore, the current study was initiated to:

1. Investigate the waterborne pathogens (*E. coli* and *P. aeruginosa*) in different water type (surface, ground and wastewater) collected from fresh produce farm.
2. Detect the occurrence of such pathogens in crops, animals and farm worker samples collected from the same farms.

3. Detect the virulence genes of these pathogens using molecular techniques.
4. Analysis the antibiotic sensitivity pattern of *P. aeruginosa*.
5. Establish the epidemiological cycle between the same isolates from different sources (water, animals, crops and human) using sequencing and phylogenetic tree.

Review of Literature

I. Water-borne zoonoses:

Bolin *et al.*, (2004) reported that world wide data indicates that about 75% of emerging infectious disease is caused by zoonotic pathogens and some research studies report that about 80% of zoonotic disease could be waterborne**.**

WHO, (2008) reported that increasing the introduction of a zoonotic pathogen to the water environment, arising from changes in control and management practices such as introducing sewage without treatment or through the land application of inadequately treated manures produced from agricultural processes. Contamination results from the discharge/runoff of untreated waste or inadequately treated wastewater into water bodies.

WHO, (2009) described the principal vehicles of transmission of waterborne zoonotic pathogens. Regarding to the water source, drinking-water; water contact; water used in food preparation, production (including agricultural produce and seafood products), and processing; and aerosolized materials might transmit such pathogens. Identification of the sources and transmission routes of waterborne zoonotic infections was essential for developing and implementing appropriate control mechanisms.

II. Microbial hazards linked to different irrigation water source:

Steele and Odumeru, (2004)found that groundwater, surface water, and human wastewater were commonly used for irrigation. The risk of disease transmission from pathogenic microorganisms present in irrigation water was influenced by the level of contamination; the persistence of pathogens in different ecosystem (water, soil and crops); and the route of exposure. They also reported that groundwater was generally of good microbial quality, unless it is contaminated with surface runoff; human wastewater was usually of very poor microbial quality and required extensive treatment before it could be used safely to irrigate crops; surface water is of variable microbial quality.

Bourquin, (2009) suggested that municipal water (treated wastewater) and ground water provided the lowest risk for irrigation purposes. However, using these water sources was often limited to field location and size. Surface water was the most common source for irrigation on fruit and vegetable farms. It was recommended that upstream neighbors should keep animals out of waterways and prevent feedlot runoff from entering streams.

Shelton *et al.*, (2011) mentioned that fecal indicator organisms were poor predictors of the potential for water to cause gastrointestinal illness.

Alam, (2014) mentioned that irrigation water was usually stored in a source, natural or artificial, prior to use. Fresh water that could be used for irrigation and which was accessible to humans comprised less