

GENETIC CHANGES IN FOOD CONTAMINANTS BACTERIA ISOLATED FROM DIFFERENT ENVIRONMENTS

BY

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B.Sc. Agric. Sc. (Poultry Production), Ain Shams University, 1985
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University, 2006

A Thesis Submitted in Partial Fulfillment
of
The Requirements for the Doctor of Philosophy
in
Environmental Science

Department of Environmental Agricultural Science
Institute of Environmental Studies & Research
Ain Shams University

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APPROVAL SHEET

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ABSTRACT

**Mohamed Abdel-Maksoud Abdel-Wahed,
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In this study, a total of 187 samples (62 chicken parts, 22 skin samples of whole chicken carcasses, 30 raw egg yolks, 30 eggshells, 15 raw milk, 7 minced meat and 21 chicken feces samples) were screened for the presence of *Salmonella*. The isolates were characterized by serogrouping, serotyping, antimicrobial-susceptibility testing and production of extended-spectrum beta-lactamases (ESBLs). The polymerase chain reaction was used to identify antibiotic resistance genes and integrons. The rates of *Salmonella* isolation were 66.1%, 50%, 6.7%, 14.2% and 62% for chicken parts, chicken carcasses, raw milk, minced meat and chicken feces respectively, whereas the egg yolks and eggshells were uniformly negative. *S. Kentucky* and *S. Enteritidis* serotypes composed 42.8% and 2.4% of the isolates, respectively, while *S. Typhimurium* was absent. Variable resistance rates were observed 97% were resistant to sulfamethoxazole; 96% to nalidixic acid and tetracycline; 75% to ampicillin, and 67% to ticarcillin/clavulanate. Multi-drug resistance was detected in 78% (66/84) of the isolates, and ESBL production was detected in 7.1% (6/84). The β -lactamase *bla*TEM-1 gene was detected in 57.6% and *bla*SHV-1 in 6.8% of food isolates, while the *bla*OXA gene was absent. The *sul1* gene was detected in 97.3% and the *sul2* gene in 5.3% of food isolates. Sixty two of the 78 isolates (79.5%) were positive for the integrase gene (*intI1*) from class 1 integrons while *intI2* was absent. The 5'- and 3'- conserved segment (CS) regions were identified in 49.1% of the *intI1* positive isolates, eight types of class 1 integrons were detected for the *Salmonella* isolates. Five *S. Kentucky* isolates resistant to ciprofloxacin carried the 1.95 kp class 1 integron.

Key Words: *Salmonella*, antimicrobial resistance, resistance mechanism, Egypt

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LIST OF ABBREVIATIONS

Term	Meaning
AM	Ampicillin
API	Analytical Profile Index
ATCC	American Type Culture Collection
ATM	Aztreonam
BPW	Buffered Peptone Water
BSA	Bismuth Sulfite Agar
bp	Base pair
CAZ	Ceftazidime
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
C	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
CTX	Cefotaxime
CO ₂	Carbon dioxide
°C	Degrees Celsius
DNA	Deoxyribonucleic acid
DT	Definitive Type
DNase	Deoxyribonuclease
dNTP	Deoxynucleotide triphosphate
dH ₂ O	Distilled water
E	Erythromycin
e.g.	<i>exempli gratia</i> , for example
<i>et al</i>	et alia, and other people
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ESBL	Extended Spectrum β -lactams
GM	Gentamicin
g	gram
xg	relative centrifugal force
GM	Gentamicin
h	hour
IMP	Imipenem
HCl	Hydrochloride acid
H ₂ O	Water

Kb	Kilobase pairs
L	Liter
LB-broth	Luria Bertani Broth
LB-agar	Luria Bertani Agar
MDR	Multidrug resistant
mg	Milligram
min	Minutes
MKKTTn	Müller Kauffmann Thetrathionate Novobiocin Broth
MLST	Multilocus Sequence Typing
NA	Nalidixic acid
NT	Non-Typable
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NOR	Norfloxacin
OD	Optical density
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
pH	negative logarithm to the base ten of the concentration of hydrogen ions
rpm	Revolutions per minute
RFLP	Restriction fragment length polymorphism
s	Seconds
S	Streptomycin
SUL	Sulphafurazole
TAE	Tris, acetate, EDTA buffer
TBE	Tris-Borate-EDTA
TBS	Tris buffer saline
TE	Tris, EDTA buffer
Tris	Tris (hydroxymethyl) amino methane
TE	Tetracycline
SXT	Trimethoprim/sulfamethoxazole
SGI1	Salmonella genomic island 1
Tm°C	Melting temperature
v/v	Volume per volume
w/v	Weight per volume
XLD	Xylose lysine deoxycholate agar
WHO	World Health Organization
µg	Microgram
µL	Microlitre

1. INTRODUCTION

Salmonella and *Campylobacter* are the most important food-borne disease etiologies, causing substantial medical and economic burdens worldwide (**Cardinale *et al.*, 2003**).

Food-borne diseases caused by non-typhoid *Salmonella* represent an important public health problem and an economic burden in many parts of the world. The main sources of infections are foods of animal origin, such as poultry, eggs, milk, beef and pork. In addition, fruits and vegetables have been implicated as vehicles in *Salmonella* transmission. In the last two decades, the emergence and spread of antimicrobial-resistant pathogens, among them *Salmonella*, has become a serious health hazard worldwide (**Miko *et al.*, 2005**).

More than 1.6 million cases of human laboratory-confirmed *Salmonella* infections were reported during 1999–2008 in 27 European countries (**EFSA, 2010b**). In high-income regions of North America, there are an estimated 1.7 million *Salmonella* infections per year, and ~2800 are fatal (**Majowicz, *et al.*, 2010**).

Campylobacter species have been identified as a major cause of bacterial gastroenteritis in humans worldwide (**Altekruse, 1999**). Depending on the country, either *Campylobacter* or *Salmonella* is the most frequently isolated bacterial pathogen diagnosed from cases of diarrhea (**Tauxe, 2002**).

The evolution, increasing prevalence and dissemination of pathogenic bacteria resistant to multiple antimicrobial agents is currently recognized as one of the most important problems in global

public health (**Bush, 2010**). The rapid spread of antibiotic resistance genes, facilitated by mobile genetic elements such as plasmids, transposons and integrons, has led to the emergence of multidrug resistant (MDR) strains of many clinically important species that now frequently leave clinicians out of therapeutic options (**Hawkey & Jones, 2009 Livermore 2009**).

In Egypt, antibiotic resistance has been reported among human *Salmonella* isolates, including *Salmonella enterica* serovar Typhi (S. Typhi) and diarrheagenic strains (**Wasfy *et al.*, 2000 and Abdelhakim *et al.*, 2011**). However, since there is no national *Salmonella* reference centre to provide reliable statistical data, little is known about foodborne *Salmonella* in Egypt.

Aim of the present study

There is still considerable lack of information with regard to (i) the prevalent serotypes, (ii) the molecular characteristics (iii) the antimicrobial patterns, and (iv) the genetic basis of antimicrobial resistance of *Salmonella* and *Campylobacter* strains isolated from foods in Egypt. The objective of study is to contribute information that will help fill the gaps in these areas of research.

The plane of the present study is outlined below:

- i) Through microbiological testing, determining contamination rates of chicken meat, feces and eggs with *Salmonella* and *Campylobacter*.
- ii) Characterizing *Salmonella* and *Campylobacter* isolates isolated from chicken meat. Isolates will be