# GENETIC CHANGES IN FOOD CONTAMINANTS BACTERIA ISOLATED FROM DIFFERENT ENVIRONMENTS

BY

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M.Sc. Agriculture Sciences (Agricultural Microbiology), Ain Shams University, 2006

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

Department of Environmental Agricultural Science
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## APPROVAL SHEET

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# ABSTRACT


**Mohamed Abdel-Maksoud Abdel-Wahed,**
**Genetic changes in food contaminants bacteria isolated from different environments. Unpublished PhD thesis, Ain Shams University, Institute of Environmental Studies & Research, Department of Agricultural Sciences, 2014.**

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 (*intl1*) from class 1 integrons while *intl2* 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

## CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

**Fig. no.** **Page no.**

## 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 |
| CO2 | Carbon dioxide |
| °C | Degrees Celsius |
| DNA | Deoxyribonucleic acid |
| DT | Definitive Type |
| DNase | Deoxyribonuclease |
| dNTP | Deoxynucleotide triphosphate |
| $dH_2O$ | Distilled water |
| E | Erythromycin |
| e.g. | *exempli gratia*, for example |
| *et al* | 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 |
| $H_2O$ | 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/sulfamethoksazol |
| 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 S*almonella* 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