



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF *SALMONELLA* SPECIES
FROM STREET FOOD AND CLINICAL SAMPLES**

TUNUNG ROBIN

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**ISOLATION AND CHARACTERIZATION OF *SALMONELLA* SPECIES
FROM STREET FOOD AND CLINICAL SAMPLES**

By

TUNUNG ROBIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

May 2007



**Dedicated to my beloved family and dear friends for their loving support
To God Almighty for the strength and wisdom**

**Fear of the Lord is the beginning of knowledge,
Fools despise wisdom and instruction**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the degree of Master of Science

**ISOLATION AND CHARACTERIZATION OF *SALMONELLA* SPECIES
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May 2007

Chairman: Associate Professor Fatimah Abu Bakar, PhD

Faculty: Food Science and Technology

The aims of this study were to isolate and characterize *Salmonella* spp. from food sampled randomly from street vendors in Selangor, Malacca, Kuala Lumpur and Negeri Sembilan, and from clinical samples in a hospital in Klang, Selangor (collected from January to September 2004). A total of 24 *Salmonella* strains, belonging to seven different serotypes, were isolated from 129 different street-vended foods and drinks and 12 rectal swabs from hospital patients suspected with salmonellosis. The most frequently encountered serotype from street foods was *Salmonella* Bialfra (66.7%) while the most frequently encountered serotype from clinical samples was *Salmonella* Typhi (66.7%). To the best of our knowledge, this is the first reported occurrence of *Salmonella* Bialfra in street foods in Malaysia. Other serotypes isolated from street foods were *Salmonella* Braenderup (25%) and *Salmonella* Weltevreden (8.3%), and from clinical samples were *Salmonella* Typhimurium (17%), *Salmonella* Paratyphi A (0.08%) and *Salmonella* Paratyphi B (0.08%). All isolates were tested for their resistance to the following 14 antibiotics: streptomycin, trimethoprim, sulphamethoxazole, tetracycline, cefuroxime,



ciprofloxacin, ampicillin, chloramphenicol, gentamicin, rifampin, penicillin, nalidixic acid, norfloxacin and erythromycin. All strains (100%) were found resistant to the antibiotic rifampin. None of the strains were however resistant to cefuroxime. A dendrogram was generated for antibiotic resistances of the isolates, and six clusters were defined, with similarity levels ranging from 18.8% to 100%. Generally, street foods and clinical isolates tend to cluster apart. A dendrogram to cluster the antibiotics was also generated, and they could be grouped according to their classes based on mode of inhibition: cell wall synthesis, protein synthesis, or nucleic acid synthesis. The most related compounds were chloramphenicol and tetracycline (46.6% similarity), which is acceptable as they share the same mode of inhibition. Ampicillin was grouped in the same cluster at 13.5% similarity although of different inhibition mode, possibly due to cross-resistance. Out of the 24 isolates screened for the presence of plasmid, 15 harbored plasmids. The plasmid sizes ranged from 3.0 to 38.5 MDa. Randomly amplified polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus (ERIC) analysis were performed for the molecular genetic typing of the strains. RAPD fingerprinting with the primers OPAR3 and OPAR8 produced a combination of 21 fingerprint patterns, while ERIC fingerprinting generated 19 patterns. Cluster analysis of *S. Bifra* showed that similar serotypes were found in different food samples collected at different locations. The likely source of this result was cross contamination during food handling. Cluster analysis of *S. Typhi* showed that all the serotypes were different, even though the location of sampling was the same. The results indicate multiple source of *S. Typhi* infection of the patients. From this study, RAPD was found to be more discriminative than ERIC.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMENCILAN DAN PENCIRIAN SPESIS *SALMONELLA* DARI SAMPEL MAKANAN GERAI DAN KLINIKAL

Oleh

TUNUNG ROBIN

Mei 2007

Pengerusi : Profesor Madya Fatimah Abu Bakar, PhD

Fakulti : Sains dan Teknologi Makanan

Matlamat kajian ini adalah untuk memencilkan dan mencirikan pencilan-pencilan spesies *Salmonella* dari sampel makanan gerai dan klinikal yang diperolehi dari lokasi rawak di Selangor, Melaka, Kuala Lumpur dan Negeri Sembilan, dan dari sampel klinikal di sebuah hospital di Klang, Selangor (dikumpulkan dari Januari hingga September 2004). Sejumlah 24 pencilan *Salmonella*, yang terdiri daripada tujuh serotip berbeza, dipencilkan daripada 129 makanan dan minuman daripada gerai yang berbeza dan 12 sampel klinikal (swab rektum). Serotip yang paling kerap dijumpai daripada makanan gerai adalah *Salmonella* Biafra (66.7%) dan daripada sampel klinikal adalah *Salmonella* Typhi (66.7%). Pada pengetahuan kami, ini merupakan laporan yang pertama tentang kehadiran *Salmonella* Biafra dalam makanan gerai di Malaysia. Serotip lain yang dipencilkan dari makanan gerai adalah *Salmonella* Braenderup (25%) dan *Salmonella* Weltevreden (8.3%), dan dari sampel klinikal adalah *Salmonella* Typhimurium (17%), *Salmonella* Paratyphi A (0.08%) dan *Salmonella* Paratyphi B (0.08%). Kerintangan pencilan-perncilan tersebut telah dinilai dengan menggunakan antibiotik berikut: streptomycin, trimethoprim,



sulfamethoxazole, tetracycline, cefuroxime, ciprofloxacin, ampicillin, chloramphenicol, gentamicin, rifampin, penicillin, nalidixic acid, norfloxacin dan erythromycin. Semua pencilan tersebut (100%) didapati rintang terhadap rifampin. Namun tiada pencilan yang rintang terhadap cefuroxime. Dendrogram kerintangan antibiotik pencilan-pencilan tersebut dihasilkan, dan enam kluster telah ditakrifkan, dengan tahap persamaan 18.8% ke 100%. Secara amnya, pencilan makanan gerai dan klinikal cenderung untuk mengkluster berasingan. Dendrogram untuk mengkluster antibiotik juga dihasilkan, dan antibiotik-antibiotik tersebut boleh dikumpulkan mengikut kelas berdasarkan mod inhibisi: inhibisi sintesis dinding sel, sintesis protein atau sintesis asid nukleik. Antibiotik yang paling berkait rapat adalah chloramphenicol dan tetracycline (persamaan 46.6%), yang mana ianya boleh diterima kerana berkongsi mod inhibisi yang sama. Ampicillin berkumpul di dalam kluster yang sama pada persamaan 13.5% walaupun mempunyai mod inhibisi yang berbeza, kemungkinan disebabkan kerintangan silang. Daripada 24 pencilan yang telah diuji untuk kehadiran plasmid, 15 pencilan mengandungi plasmid. Saiz plasmid berada dalam lingkungan 3.0 ke 38.5 MDa. Analisis amplifikasi polimorfik DNA rawak (RAPD) dan konsensus intergenik repetitif enterobakterial (ERIC) telah dilaksanakan untuk mengetip genetik molekular pencilan-pencilan tersebut. RAPD dengan OPAR3 dan OPAR8 menghasilkan kombinasi 21 pola, sementara ERIC menghasilkan 19 paten. Analisis kluster *S. Bifara* menunjukkan bahawa serotip yang sama dijumpai daripada sampel makanan gerai yang berbeza daripada lokasi yang berbeza. Kemungkinan ini adalah kerana kontaminasi silang semasa pengendalian makanan. Analisis *S. Typhi* menunjukkan bahawa serotip adalah berbeza walaupun lokasi sampel sama. Keputusan tersebut menunjukkan sumber jangkitan *S. Typhi*

yang berlainan pada pesakit-pesakit tersebut. Melalui kajian ini, RAPD adalah lebih diskriminatif berbanding ERIC.

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I certify that an Examination Committee has met on 29th May 2007 to conduct the final examination of Tunung Robin on her Master of Science thesis entitled “Isolation and Characterization of *Salmonella* species from Street Food and Clinical Samples” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

TUNUNG ROBIN

Date: 10 August 2007



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LIST OF ABBREVIATIONS

AP-PCR	arbitrary primered polymerase chain reaction
BGA	brilliant green agar
BPW	buffered peptone water
BSA	Bismuth Sulphite Agar
CDC	Centers for Disease Control and Prevention
DNA	deoxyribonucleic acid
EC	European Commission
EDTA	ethylenediaminetetra-acetic acid
EMBA	eosin-methylene blue agar
ERIC	enterobacterial repetitive intergenic consensus
FAO	Food and Agriculture Organization
FDA	Food Drug Administration
FSRI	Food Safety Research Institute
GET	glucose-EDTA-Tris
H ₂ S	hydrogen sulphide
HEA	Hektoen Enteric agar
kb	kilo basepair
LB	Luria-Bertani
MA	MacConkey agar
MAR	multiple antibiotic resistance
MDa	mega Dalton
MOH	Ministry of Health



NCCLS	National Committee for Clinical Laboratory Standards
NST	New Straits times
PCR	polymerase chain reaction
RAPD	randomly amplified polymorphic DNA
rpm	revolution per minute
RV	Rappaport-Vassiliadis
SDS	sodium dodecyl sulphate
SSA	<i>Salmonella-Shigella</i> agar
TBE	Tris-Borate-EDTA
TSI	triple sugar iron
TS	The Star
UV	ultra violet
V	volts
WHO	World Health Organization
x g	unit gravity
XLD	xylose lysine deoxycholate



CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Food borne illness is a major international public health concern (Cardinale *et al.*, 2005) and was estimated to be the cause of 76 million illnesses, 325,000 hospitalizations, and 5000 deaths in the United States annually (Mead *et al.*, 1999; Badrie *et al.*, 2005). According to the World Health Organization (WHO, 2005), 90% of the annual deaths from food borne illnesses are among children particularly in developing countries. The WHO defines food borne illnesses as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food. Food borne illnesses remain widespread but their impact on public health has not been well documented, especially in developing countries (King *et al.*, 2000).

Many countries reported contamination of food to be the major factor contributing to food borne illness. Several countries have reported high bacterial counts in foods when microbiological studies were carried out on street-food vending (Kubheka *et al.*, 2000). According to WHO, street food refers to food and beverages prepared and sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (WHO, 2005). In developing countries, the street food trade has evolved as a result of rapid

urbanization and is known as a phenomenon that has great economical, socio-cultural, and sanitary importance (Lucca *et al.*, 2006). Street-food vendors play an important economic role as they provide a source of inexpensive, nutritious meals to a large number of office and construction workers, and to people in transit. The vendors themselves benefit from positive cash flow, as they are able to establish business with minimum capital and do not pay tax on their earning.

The usual congregation conditions for vendors (such as overcrowded areas, limited access to basic sanitary facilities and large amounts of garbage) provide harborage for insects and animal pests. In many incidences consumers are concerned about the price of food rather than its safety and hygiene. This has given rise to concerns regarding the sanitary standards of street vending operations. The main factor that currently compromises food quality is the hygienic-sanitary aspect (in which the sale of food in public places is highly controversial from a health standpoint). This practice represents a serious threat to consumer health and is a matter of great importance for public health (Lucca *et al.*, 2006). Nonetheless, food borne illnesses remain responsible for morbidity and mortality in the general population, particularly infants and children, the elderly and immunocompromised.

One of the most common causes of food borne illness worldwide is *Salmonella* infection or salmonellosis (Cardinale *et al.*, 2005). *Salmonella* is considered to be the cause of the largest number of outbreaks, cases, and fatalities that result from food borne infections among enteric pathogens. Salmonellosis is caused by the two species of *Salmonella*; *Salmonella enterica* and *Salmonella bongori*, and it is an



infectious disease of humans and animals. Investigations in developed countries have shown that *Salmonella* infections are serious in frequency and severity of symptoms (Cardinale *et al.*, 2005). Clinical symptoms of human salmonellosis include acute onset fever, abdominal pain, diarrhea, nausea and vomiting, while dehydration can become life threatening.

The main source of salmonellosis is usually contaminated food, especially poultry, poultry products, cattle and dairy products (Rychlik *et al.*, 2000). During the last decade, several salmonellosis outbreaks have been documented worldwide due to the consumption of contaminated food products or water with *Salmonella* (Llewellyn *et al.*, 1998; Harakeh *et al.*, 2004). In developing countries, street foods in particular have been reported to be contaminated with *Salmonella* and have been implicated in a few outbreaks of food borne diseases (Mankee *et al.*, 2003). *Salmonella* is one of the most frequently isolated bacteria from food, and *Salmonella* species have been isolated from a variety of foods such as milk, beef, pork, and chicken (Lim *et al.*, 2005). In Malaysia, the Ministry of Health Malaysia has reported that the food poisoning cases associated with *Salmonella* spp. were 2.3% (Shobirin *et al.*, 2003).

The incidence of food borne infections caused by *Salmonella* species has increased dramatically during the past few years. Mrema *et al.* (2004) reported that in the United States, salmonellosis is estimated to affect 1.4 million people each year, and 95% of the cases are foodborne. Outbreaks of *Salmonella* food poisoning has become the most frequent health problem in Japan, as indicated by the number of cases reported and the number of patients affected. In Malaysia, an outbreak of